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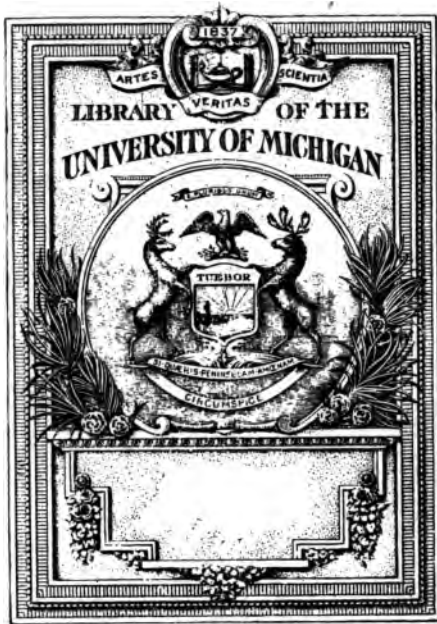
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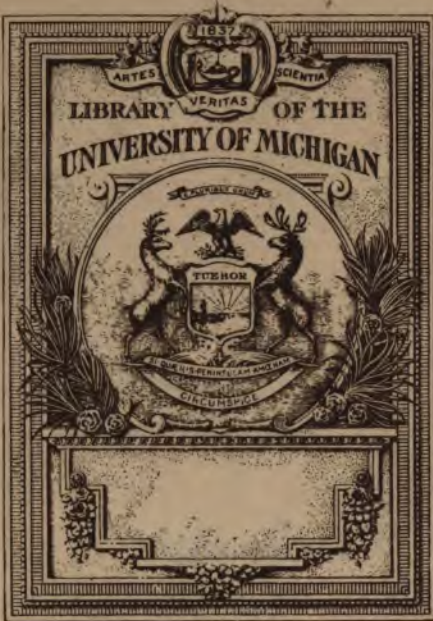
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04

THE GIFT OF
Jackson High School

Francis Lenton



THE GIFT OF
Jackson High School

Francis Lantieri







MICROSCOPIC BOTANY.

A MANUAL OF THE MICROSCOPE

IN



VEGETABLE HISTOLOGY.

BY

DR. EDUARD STRASBURGER.

FROM THE GERMAN

BY

REV. A. B. HERVEY.

BOSTON:
SAMUEL E. CASSINO,
1887.

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TRANSLATOR'S PREFACE.

The "Kleine Botanische Practicum" of which this is a translation is an abridgment of a larger work of the same kind made by the author, Dr. Strasburger. I have made further condensation of the work in those chapters where the matter and expression would admit, notably in Chapter VIII and those immediately preceding, and in Chapters XXX and XXXI, but in no case have I omitted essential matters from the text. The Introduction alone is shortened by omission, because portions of it were quite irrelevant to the purposes of an American edition. For a similar reason, and to save space, the Author's preface is not reproduced.

The first two "Registers" or Indices, those enumerating the plants and reagents used in the studies, are also omitted, as being neither essential nor very important, the references being all contained in the general subject-index. The formulæ contained in register II are found in an appendix.

The work is divided into thirty-two Lessons or Chapters to adapt it to the weeks in a German College year. It is believed that the work is well adapted to the needs of both the solitary worker and students in American Colleges.

A. B. HERVEY.

Taunton, Mass., 4 July, 1887.

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INTRODUCTION.*

IN case the beginner should wish to provide himself with a water-immersion lens, he is recommended to get one without the screw-collar adjustment. He will find it more difficult to learn the management of the other sort. The most skilful observers make no use of this adjustment with the lower powers, and these are the ones referred to here.

Immersion lenses without the screw-collar adjustment are corrected for a given thickness of cover-glass and should be used with that thickness only. By providing himself with that glass he can dispense with the correction adjustment even with his high powers, and would need it only in studying objects already mounted under cover-glasses of other thicknesses.

He who is not afraid of a larger expenditure will do well to buy a "homogeneous" instead of a water-immersion system. These systems are all without collar correction since the thickness of cover-glass within permissible limits is a matter of indifference. Since they will bear much higher eye-pieces than either the dry or the water-immersion

*A considerable part of the Author's Introduction is given up to the mere enumeration of stands and lenses of German and other Continental and English makers, compiled from their latest catalogues. As American students would mostly buy American instruments, or could easily get the same information from the catalogues used by the author, it was not thought necessary to print this part of the text. The main import of his teaching on this point is to choose the simplest and handiest stand, with lenses of medium power; such as in this country are generally known as "Students' Microscopes." The stands should however be chosen with reference to the use of the higher and highest objectives on them. Speaking of water-immersion lenses he continues as above.—A. B. H.

sion systems, as many different magnifications may be got with one of these as with several of the latter. Homogeneous immersion lenses give the best results when used with the Abbé illuminating apparatus. This apparatus is applied only to the larger and more costly stands. * * * Still the homogeneous systems may be used with great advantage on the smaller stands.

It does not come within my purpose to propound a theory of the production of the microscopic image, but refer my readers to text-books of physics and special works on the microscope for that (1). It is my aim to make the beginner familiar with the more important facts of microscopical botany, with the use of the microscope and with microscopical technique. This can be accomplished only by study. But for convenience in comparing and consulting the various statements scattered through the text a detailed index is appended.

Besides the compound microscope, a simple, or a so-called "preparing microscope" or "simplex," is also necessary. For all the purposes contemplated in this book, the small preparing stand (No. 111 of Zeiss' Catalogue, for \$4.50) with a magnifying glass having a power of from five to ten diameters (No. 112) for \$1.50, a doublet of fifteen and another of thirty diameters of magnification (No. 113) for \$1.50 each, are sufficient. The magnifying glass employed in this combination may also be used as a hand magnifier.

The compound microscope may be used for a "preparing microscope" by uniting a prism with the ocular as in Nachtet's plan, or by an "erecting" ocular like that of Hartnack. For the use of this contrivance a draw-tube is necessary, the erecting prism being screwed into the lower end of it. The image loses something of its sharpness but still sufficiently answers its purpose. A certain advantage

is gained in manipulating very small objects under the compound microscope, for they are not lost from the field of view, and do not have to be transferred from the compound to the simple microscope and back again to find them. Working with the erecting ocular offers scarcely greater difficulties than with a "simplex," but the erecting prism obliges one to look obliquely forward rather than downward toward the hands, which is somewhat inconvenient. The erecting prism attached to the ocular diminishes the field of vision in most cases. Very low powers must be used for this work.

Another indispensable requisite is a good magnifying glass by which to make our preliminary survey of the object to be investigated. As before mentioned, if the preparing microscope is furnished with a good magnifier it may be used as a hand-lens. One with a power of about six diameters is usually preferred. The aplanatic magnifiers are very excellent and correspondingly expensive.

For a drawing prism to be used on the microscope, either the new Abbé camera lucida (Zeiss Cat., No. 64), or the camera lucida with two prisms (Zeiss Cat., No. 65), is to be preferred before all others. The former is fitted and adjusted to the No. 2 ocular. During observation it is removed. It allows one to draw on an horizontal surface. The latter is fastened to the tube or ocular with a ring. The drawing is done on an inclined surface. It may be kept constantly on the microscope, being turned to one side during observation. The drawing table, either horizontal or inclined at an angle of 25° as the case may be, should be adjusted at the height of the microscope stage, or at the distance of clearest vision for an especially near- or far-sighted observer.*

One needs also an objective micrometer.

*It should always be fixed at a standard distance of ten inches from the focus of the ocular.—A. B. H.

Any steady work-table may be used in microscopical work. It should be neither too small, nor have a bright surface. Choose a window with a free outlook, if possible (it matters not in what direction), and place the microscope $1\frac{1}{2}$ to 2m. from it. If there is direct sunlight, interpose a white curtain. This glaring white light is the best possible for working with high powers.

Object-slides and cover-glasses may be obtained of the dealers. As between the Giessen form of object-slide, which is 48 mm. long by 28 mm. wide, and the English which is 76 by 26 mm., the latter is in many respects handier. Of the cover-glasses, those for common use may

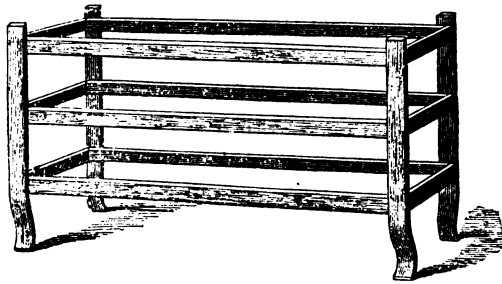


FIG. 1. Zinc rack for slides. Used under glass bell.

conveniently be squares about 18 mm. on a side, but for special purposes both smaller and larger forms may be provided. If one uses high powers, he should have his cover-glasses prepared of a definite thickness.*

One will also need razors both flat and concave; small and large steel forceps; fine-pointed dissecting scissors, for which fine embroidery scissors will answer; a pair of needle holders somewhat like a crochet-needle holder, but one in which the finest sewing needle may be held fast; English sewing needles from No. 8 upwards; scalpels; fine

* For ordinary use, circles are to be preferred to squares. Those 18 mm. in diameter are perhaps best for examination of objects in water. But for permanent preparation of small objects, circles of uniform size, 15 mm. in diameter should be chosen.—A. B. H.

camel's hair pencils ; a small, watchmaker's hand-vice ; glass tubes and rods ; watch glasses of different sizes, and glass disks of corresponding sizes with which to cover them ; low glass bells with which to construct moist-chambers ; zinc racks as illustrated, about one-half natural size in Fig. 1, on which to lay the slides under the bells ; two high glass bells with which to cover the simple and compound microscopes ; and, finally, elder pith.

The list of necessary reagents is appended at the end of this book.

For the preservation of permanent preparations, the dealers in such goods furnish excellent cabinets, in which the objects lie flat, and can easily be found and inspected.

NOTE.

(1) Having special reference to Botanists. Naegeli und Schwendener, *das Mikroskop.*, 2 Aufl. 1877. Dippel, *das Mikroskop.*, 2 Aufl. 1882. Behren's *Helfsbuch*, etc., 1883. American Edition, Hervey. S. E. Cassino & Co., 1885.

LESSON I.

USE OF THE MICROSCOPE. STRUCTURE OF STARCH.

THE separate parts of the Compound Microscope, as seen in the Zeiss stand No. VIIA Fig. 2, are as follows: the foot *fs*; the column *sl'*; the stage *ot*; the spring sheath *fh*; the tube *t*; the mirror *s*, concave on one side and plane on the other; the former should be used with high and the latter with low powers. The stage has a circular opening for the passage of the light from the mirror. Beneath this is a cylinder diaphragm *cb*, fixed in a slide which shoves into the stage from the side.

The cylinder diaphragms have openings of different sizes and are movable up and down in an outer cylinder, or holder, attached to the slide. It should be first barely inserted in the holder, and after that has been pushed into place, it should then be raised sufficiently to be even with the top of the stage. The amount of light used is regulated by the diaphragm, but in the beginning it is best to leave the diaphragm out. Zeiss' stands, Nos. VIIb and VIII, have for diaphragms hemispherically-shaped disks, eccentrically pivoted and provided with openings of various sizes which, by revolving the disk, successively come within the optical axis of the microscope.* The stage is provided with removable spring clips, *fil*, for holding the object-slide in place. The tube, *t*, is movable in the spring sheath *fh*. In the larger stands the tube is moved with a rack and pinion, without the sheath.

* American Microscopes have also diaphragms of flat disks thus perforated and mounted.—A. B. H.

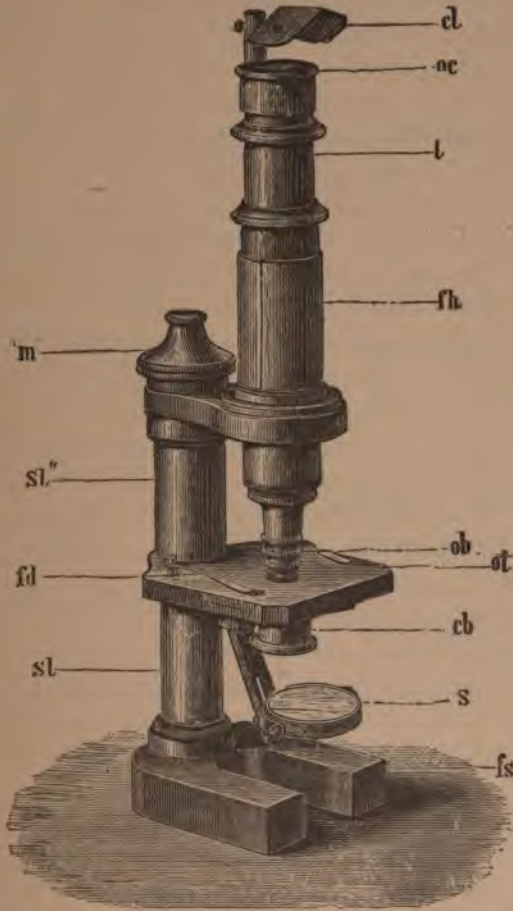


FIG. 2. Stand VIIa of Zeiss with drawing prism $\frac{1}{2}$ natural size. *fs.* foot; *sl'*, under, and *sl''*, upper part of column; *ot*, stage; *cb*, cylinder diaphragm; *fd*, spring-clips; *s*, mirror; *m*, fine adjustment screw; *fh*, spring-sheath; *t*, tube; *ob*, objective; *oc*, ocular.

Into the lower end of the tube, screw a low-power objective and set one of the weaker oculars, without the camera lucida, *cl*, into the upper end. Placing the microscope before a window and looking into the ocular, ad-

just the mirror so that the field of view in the microscope will be brightly and uniformly illuminated. For direct illumination place the mirror in the optical axis of the microscope as it is not in the illustration. The quantity of light may be regulated by moving the mirror up or down upon its carrier within the optical axis.

Upon a clean object-slide put a small drop of pure water. Cut a potato in two with a pocket knife and put some of the liquid which exudes from the cut surface into the water drop, and place over it a clean cover-glass.

The cover-glass may be cleaned by holding and rubbing it flatwise between the fingers with a piece of old linen. If the drop of water should be too large it will run out beyond the edge of the cover-glass, in which case the superfluous water may be taken up with a piece of blotting paper or cloth. It is better, however, to make a new preparation; for the action of the blotting paper, in soaking up the superfluous water, will draw out a great part of the starch grains from under the cover-glass.

Place the preparation on the stage directly over the opening. Push the tube down till the objective nearly touches the object, guiding the movement with the eye looking across the stage from the side. Now look into the ocular and draw the tube very slowly upwards with a rotary motion. Soon, the previously invisible objects will appear in the form of small grains. If they do not, and the lens is drawn back 2 cm. or more from the object, we may conclude either that the object is not in the field of view, or that we have drawn the tube back so quickly that the image has been so rapidly formed and dissipated as to escape observation. But, to find the image, do not run the tube down in search of it, else we shall be in danger of pushing the lens down upon the object, crushing the cover-glass, ruining the object and soiling the lens.

Rather proceed as at first, only drawing the tube back more slowly and carefully.

If the object is not really in the field, put it there by moving the slide, and find it. Having found the grains, the more exact focussing may be finished by means of the micrometer screw *m*, Fig. 2. This should be turned experimentally either way, the adjustment being perfect only when the image appears with the utmost possible distinctness. With the larger stands the coarse adjustment is done with the rack and pinion and not with the hand.

Having made sure of the existence of the small grains on the slide in the field of the microscope, we notice the distance which the objective is from the object, and note this as a guide for its future use; then, leaving the object on the stage, we replace the low-power lens with a higher power but not with an immersion lens. Push the tube down till the lens almost touches the cover-glass and focus as before, remembering to draw the tube upwards all the more slowly the higher the power of the lens. Directly the grains become visible, finish the focussing with the fine-adjustment screw. Notice now the shorter distance of the lens from the object.

The real investigation now begins. If the two eyes are equally good, the beginner would do well to accustom himself to observe with the left eye. This will leave the right eye free to be used in drawing, while he continues to observe with the left one. The beginner should always keep the eye not in use open. At first his attention will be distracted by surrounding objects, but he will soon overcome this difficulty and be able to concentrate his whole attention on the object seen in the microscope to the exclusion of all others.

We easily see that the granules in the field of view are solid and distinctly laminated. They are starch grains.

Move the slide till a point is found where the grains lie a little separated from each other, and select for particular examination such grains as show the most distinct lamination. Every movement communicated to the object by the hand will be, apparently, greatly increased, and exactly reversed in the field of the microscope. The difficulties

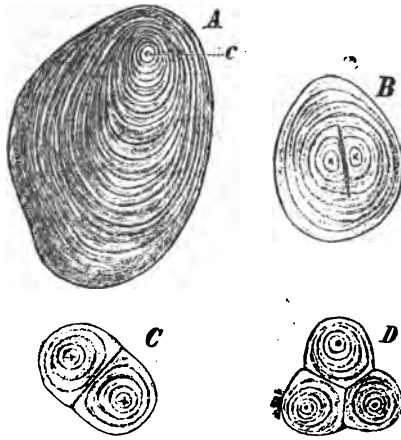


FIG. 3. Starch grains from the potato. *A*, simple grain; *B*, a semi-compound grain; *C* and *D*, wholly compound grains; *c*, nucleus. $\times 540$.

arising from this will be overcome only by practice.

Having selected a favorable grain for examination, replace the ocular with a stronger one. The light will be diminished but the image should remain distinct. Readjust the mirror so as to get as much light as possible. If, when moving or adjusting the preparation, we find the image become suddenly indistinct, we shall probably find it to be caused by the fluid from the preparation coming in contact with the lower lens of the objective. This might easily happen if too much fluid were used and so should exude around the edge of the cover-glass. The lens must be wiped dry with a piece of clean linen, or preferably with a newly-cut piece of elder pith.

Potato starch-grains (1) are relatively large and are eccentrically formed: *i. e.*, their organic middle point *c*, Fig. 3, *A*, is not in the geometric centre but nearer one end of the grain. The layers are not uniformly distinct; but between those strongly marked are others much less distinct, as they are also towards the edge of the grain. The organic nucleus on account of its great thinness is rose-colored. It is most distinctly so where it is hollowed out. It then appears as a rosy point, or mark, star or cross, with a dark outline. The layers immediately surrounding the nucleus are concentrically developed, but the eccentricity soon develops and the layers thin out towards one end in such a way as to make the grain in this direction quite wedge-shaped. On the less fully developed portion of the grain which we will call the anterior end, the lamination appears more indistinct on account of its being at a less distance from the surface. The individual grains vary in size, form and distinctness of lamination. Air bubbles often appear in the fluid used in the preparation, and may be known by their small round bright centre, and their broad dark outer belt, the latter being darker towards the centre, and gray, interspersed with bright rings, towards the circumference. This peculiar appearance arises from the refraction and dispersion of the rays that come through the bubble, except the central ones, by passing from the denser to the rarer medium.

By focussing upon the lower part of the bubble the distinctness and brightness of the middle disk is increased but its size is diminished, while the breadth of the surrounding black belt is increased. In focussing upon the top of the air bubble the central disk is increased in size but diminished in brightness; gray rings of various shades gather about it and the surrounding border becomes smaller.

Having chosen a beautifully laminated grain, the observer should proceed to draw it. Drawing is of the first importance in microscopical investigation. We first learn to see an object clearly when we observe it with that concentration of attention necessary to its graphical reproduction. Drawing guards against superficial or cursory observation requires a substantial and thorough study of the image and, more than anything else, sharpens our observing faculty. The beginner should first draw the object at free hand. If he has not already the skill necessary to this, a little practice will give it. The object should not be drawn too small even when it seems very minute. A correct judgment of its real size in the field of the microscope is not easily acquired, hence it is well to draw it too large even, that the details of the observation may be put in. Not less important is it to provide the individual parts of the image with corresponding designations and note the name of the plant, the object, and the most important results of the observation.

By cautiously pressing upon one edge of the cover-glass with a needle, the starch grains are set rolling and we see that they are somewhat flattened. Lamination is scarcely discernible in the smallest grains.

Together with the simple grains, *A*, Fig. 3, are half-compound grains also, *B*, Fig. 3. These contain seldom more than two organic nuclei. Each nucleus is surrounded with a number of individual layers, both together by several common layers. Frequently, the two systems are separated by a cleft which cuts down to the common layers, *B*. The number of layers of each kind vary much.

The wholly-compound grains which are more common than the half-compound consist of two, *C*, rarely of three, *D*, very seldom of more than three granules. Unlike the others they have no common layers. The granules turn

their elongated and most fully-developed sides towards each other, and the division line between the granules often widens into a cleft towards the inside.

For purposes of comparison make also a preparation from air-dried potato starch. Put a trace of the starch into a drop of water as before. Before replacing the first preparation with this one on the microscope stage, withdraw the tube somewhat and raise the lens away from the object. Put the first preparation in the moist-chamber marked so as to identify it at any time later on. The moist-chamber consists of a deep plate and a glass bell. On the plate stands the zinc rack, Fig. 1, on which the preparation is laid. Water sufficient to immerse the bottom of the glass bell is poured into the plate. If the water under the cover-glass is partly evaporated, put a drop on the slide at the edge of the cover-glass and it will run in under. When we have focussed the new preparation, we find that the lamination of the air-dried starch grains is at least as distinct as that of the fresh ones. This preparation should also be put in the moist-chamber.

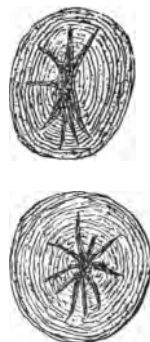


FIG. 4. Starch grains from the cotyledon of the bean *Phaseolus vulgaris*. $\times 540$.

We will now make a preparation of air-dried bean meal, *Phaseolus vulgaris*, in water. The grains, Fig. 4, are circular or oval, a little flattened, of a definite medium size. The lamination is very uniform and distinct, the structure concentric, the nucleus concave, elongated somewhat in the oval forms, and from which radial clefts extend outward nearly to the edge of the grain.

If a preparation be made with glycerine the grains will appear smaller than in water. No trace of the lamination inner cavity or clefts can be seen, these appearances being caused by the water which somewhat swells the grains.

Make a preparation of East India arrow-root starch, the

commercial article *Curcuma leucorrhiza*. If one really has the genuine, the grains will show a very eccentric structure, Fig. 5 *A*, contracted at the anterior end, very flat, and beautifully and regularly laminated. Often a number of the grains attach themselves by their flat sides and when seen from the edge look not unlike a roll of coins, *B*. The size and form of the grains do not vary much.

The West India arrow-root obtained from the rhizoma of the *Maranta*, mostly from *Maranta arundinacea*, is readily found in the market, but is much less interesting than the East India arrow-root. In water the grains resemble those from the potato, only being a little less distinctly and therefore uniformly laminated; somewhat rounder, smaller and more uniform in size. In place of the nucleus one finds a cleft in the form of a wide open V.

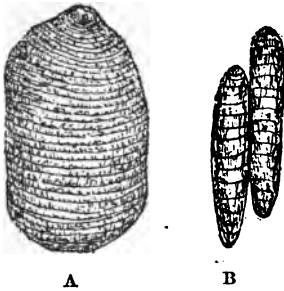


FIG. 5. Starch grains from the commercial East India arrow-root, *Curcuma leucorrhiza*. *A*, side view; *B*, view of the edges of two adhering grains. $\times 540$.

Wheat starch shows the lamination very poorly. Split a kernel of *Triticum durum* in two with a pocket knife and scrape off a little substance from the newly-cut surface into a drop of water. The large grains are circular, flattened, disk-shaped and regularly laminated, Fig. 6, *A*; still the layers are difficult to see. But, in many grains they and the nucleus may be distinctly recognized. Small grains also with distinct rose-colored nuclei, but without lamination, will be seen, Fig. 6, *B*. In many preparations the compound grains are common; but in most are not found, the component granules having fallen apart.

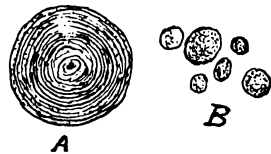


FIG. 6. Starch grains from wheat, *Triticum durum*. *A*, large, *B*, small grains. $\times 540$.

We will now halve an oat kernel, *Avena sativa*, and put a little of the substance in water. We now have the compound grain in its greatest perfection, Fig. 7, A. The size of the compound grains and the number of the component granules greatly differ. Fig. 7, A, represents one of medium size. The individual granules are polygonal and are separated by a clear boundary line. We also find smaller ones consisting of but two or three granules, and numerous single granules made by breaking up the grains. Lamination is not to be seen, and the nuclei are but rarely discernible.

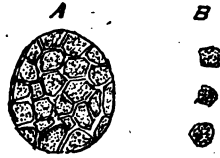


FIG. 7. Starch grains from the Oat, *Avena sativa*. A, compound grain; B, component parts of the same. $\times 510$.

The starch grains of the *Euphorbia* have a peculiar appearance. Cut a piece at will from the *Euphorbia helioscopia* and dip the cut surface into a drop of water on the slide. The milk-sap which runs out will mingle with the water and we shall see in it small isolated rod-like bodies,

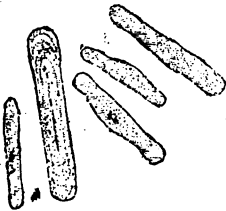


Fig. 8. Starch grains from the milk-sap of *Euphorbia helioscopia*. $\times 510$.

Fig. 8. They are the starch grains in question. They are strongly refractive; lamination is only imperfectly hinted at in the most favorable cases; in many instances a longitudinal slit is to be seen in the inside of the grain. The size of the grain varies and many appear to be smaller in the middle.

In the tropical *Euphorbia*, the starch grains are much more interesting. Make a preparation in the same manner from *Euphorbia splendens*, a plant often found in greenhouses.

The starch grains look like bones, Fig. 9. They some-

times show lamination. Sometimes a colorless sac appears on the lateral surface of the grain, *A*, the walls of which however arise not from the substance of the starch grain, but from the adhering plasma-mass.

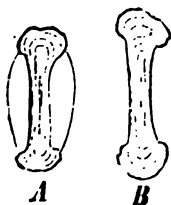


FIG. 9. Starch grains from the milk-sap of *Euphorbia splendens*. *A* is partly enveloped in a thin membrane. $\times 540$.

The small globules of the milk-sap, which are distributed through the water, are seen to be in constant, rapid, trembling motion. It is the so-called Brownian molecular movement, and is not an indication of life, but is perhaps caused by currents in the fluid which move the granules.

Having made this general survey of the form and structure of starch grains, we will now study the effects of reagents upon them under the microscope. Take the potato starch preparation; and, after focussing it, apply a drop of iodine solution to the edge of the cover-glass (iodine water, iodine alcohol, or potassium iodide of iodine). Be very careful and not get a drop on the cover-glass or the lens. If we do we must clean it at once.

Select a place not too far from the edge of the cover-glass, where the solution was applied, and then move the slide to follow the progress of the reaction. Directly the grains will change to a bright blue and then rapidly darken to a black blue. At first the lamination becomes more distinct but rapidly disappears as the grain grows opaque. A larger quantity of potassium iodide of iodine at last produces a dark brown color in the grains. Dry starch turns brown in the fumes of iodine, but water added rapidly changes it to blue. Touching the opposite edge of the cover-glass with blotting paper will hasten the movement of the reagent.

The blue reaction with iodine proves that the rod-like grains of the *Euphorbia* are really starch, notwithstanding their strange form and lack of lamination.

Let us study the action of potassium hydroxide on starch grains. Use the potato-starch, introduce the reagent and watch its effect as before. The reaction should be *very* gradual if possible. We shall first see the lamination most distinctly, and then it will rapidly disappear as the grain swells. During this swelling the nucleus is greatly hollowed out and the walls of the anterior part of the grain are folded into the cavity. Gradually the grain becomes a transparent mass with quite indistinct outline.

Finally, we should study the effect of heat on the grains of starch. Warm the preparation over a flame till it reaches a temperature of 70 C., taking care that the water is kept in it, when it will be found that the grains are swollen in exactly the same way as when treated with potassium hydroxide.

Before putting the microscope away, clean the lenses as already directed, and rub the tube and the inside of the sheath with a cloth. Cover the instrument with a glass bell which should be bound under the edge with felt.

NOTES.

(1) Compare Naegeli, Die Stärkekörner, in Pflanzenphysiol. Untersuchungen Heft 2: E. Strásburger, Bau u. Wachstum der Zellhäute, p. 107. There is also other literature.

LESSON II.

GLUTEN MEAL. FATTY OILS. MAKING PERMANENT PREPARATIONS. USE OF THE SIMPLE MICROSCOPE.

WITH a stout pocket knife bisect the cotyledons of a ripe pea, *Pisum sativum*. With a sharp razor take a thin section from the cut surface. In cutting sections with a razor observe: 1. The surface to be cut should be moistened with the fluid in which the section is to be examined, water or glycerine, —in this case with the latter. 2. The first section is not to be used since the tissue is more or less torn by the pocket knife. 3. In cutting hard tissue like this, very small sections of the utmost possible tenuity should be made, care being taken not to cut deep lest the edge of the razor be nicked. 4. Lay the edge of the razor upon the prepared surface and cut from the middle outward towards the side of the object. 5. In cutting, draw the razor in the direction of its length as well as push it forward. Both hands may be supported and steadied against the breast and yet have a sufficiently free movement. The back of the blade should rest upon the forefinger of the hand holding the object. 6. So small and hard an object as the half of a pea, and one so difficult to hold firmly in the hand should be held in a small hand-vice. 7. Be not content with a single section but make a considerable number and then select the best for the investigation.

The section should be examined in strong glycerine or with glycerine and one-third distilled water. Pure water will not do, as it causes the appearance of disorganization in the fundamental substances of the cells. Use a hair

pencil to transfer the section from the knife to the slide. Press the pencil down upon it, in taking it from the knife, which will prevent it rolling up, as it often does when seized at the edge with the forceps. Lay the section carefully in a drop of the liquid on the slide and withdraw the pencil, turning it laterally in the fingers a little at the same time.

To turn the section over upon the slide, press the pencil down upon the slide so that it will touch the edge of the section and then rotate it in a direction away from the section. This will draw the section over upon the upper side of the pencil, when it may be put down again upon the slide the other side up. Wash the pencil after using.

Put the section of the pea under a moderately high magnifying power. The tissue seems to be composed of rounded cells, Fig. 10. At the place where these cells meet is a triangular intercellular space filled with air. It is black like the edge of an air bubble. The walls of the cells are pretty thick. In each of the cells are large starch grains, also small granules, *al*, lying between. The latter are, in their turn, embedded in a very fine granular substance, *p*. At thin places in the section the starch grains are fallen out and we see the corresponding cavities in this fine, granular mass. The small grains are gluten meal, aleuron, or proteid grains (1). They lie in

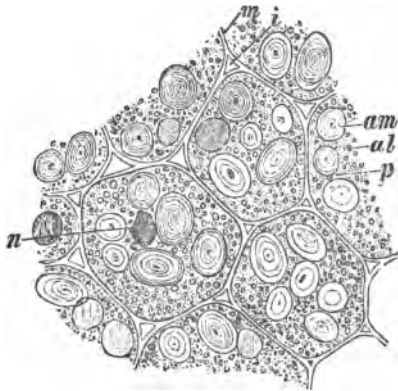


FIG. 10. Section of cotyledon of the pea. *m*, cell membrane; *i*, intercellular space; *am*, starch; *al*, aleuron grains; *p*, fundamental substance; *n*, nucleus, the last made visible only by staining. $\times 240$.

the fundamental substance of the cells. Apply an iodine solution to the section, and the various elements will assume their characteristic colors. Lift up the cover-glass and put a drop of the iodine directly on the section. The starch grains are colored blue or violet; the aleuron grains and the fundamental substance yellow. With potassium iodide of iodine the color of the latter elements becomes very intense, but the starch grains are over colored and become a dark brown. Put a section in a drop of borax-carmine solution, and in a short time the fundamental substance, and directly also the aleuron grains, will be colored a dark red, while the starch grains remain colorless. The reaction is more evident, if we replace the solution with water or dilute glycerine. This may be done by drawing out the carmine by means of blotting paper, while at the same time the other liquid is supplied at the other side of the cover-glass. A drop of Millon's reagent causes the starch grains to swell up very large and soon to become unrecognizable. But the aleuron and fundamental substance is disorganized, and the mass is colored a brick red, after a while.

If now we lay a section in a solution of methyl-green and acetic acid, there will appear in a short time, in each cell, between the other elements, a green-blue fleck of somewhat indefinite outline. This is the nucleus, *n*. The other substances have not been colored. The starch grains are a little swollen and show radial clefts. The aleuron grains are a little enlarged and appear porous or hollow. The methyl-green acetic acid is thus a specific nucleus-stain for the case in hand. The cell walls have also been stained a beautiful, bright blue color and are much more distinctly seen, as are also the intercellular spaces, than in the glycerine preparation.

Thus, we have learned to recognize albuminous sub-

stances, for such are the aleuron grains and the protoplasm (cell plasma and nucleus), by the yellow-brown reaction of iodine, the absorption of coloring matter and the brick-red reaction of Millon's reagent. We shall learn by and by that protoplasm will show these reactions only when it is dead. The reagents in this case have killed it. The nucleus shows a particularly strong affinity for coloring matter.

For our second example, selecting a kernel of wheat, *Triticum vulgare*, we first cut the kernel across in halves and make one-half fast in a hand-vice. Moisten with glycerine and cut the section quite to the outer surface of the kernel. The section examined in the same fluid will appear as in Fig. 11. Beneath the compressed dead cells of the skin, *p*, which represents the shell of fruits and seeds, lies a layer of quadrangular cells closely packed with aleuron grains. Next to this are elongated, less uniform cells which contain starch grains of all sizes. These points are all established by means of the proper reagents.

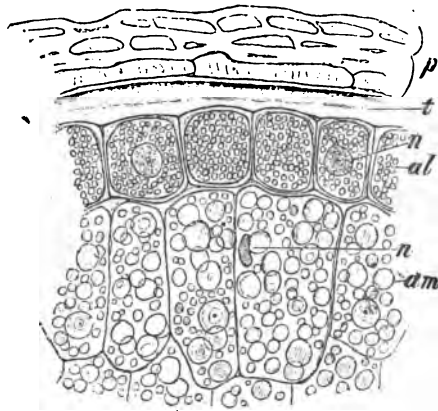


FIG. 11. Section of a grain of wheat, *Triticum vulgare*. *p*, outer, *t*, inner seed-coat; *al*, aleuron; *am* starch-grains; *n*, nucleus. $\times 240$.

Selecting a good section, we will now proceed to make a permanent preparation. We will mount our first object in the simplest possible way and one suitable to the pres-

ent case, viz., in glycerine jelly. Put as much of the jelly-like substance on the centre of the slide as we judge will make a small drop. Then warm the slide over a flame

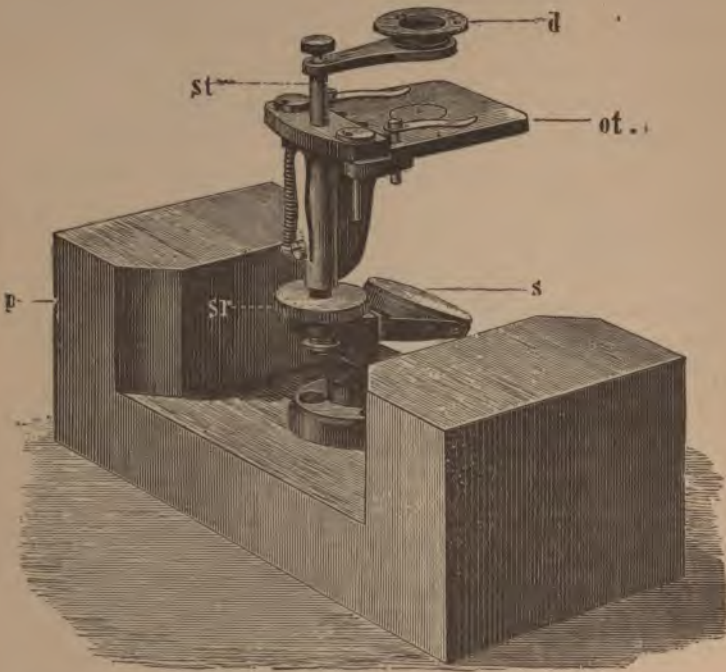


FIG. 12. Smaller preparing microscope and hand rest of Zeiss, $\frac{2}{3}$ natural size. *ot*, stage; *d*, doublet; *st*, movable rod; *sr*, fine adjustment screw; *s*, mirror; *P*, back of hand-rest.

slowly till the jelly is quite liquefied. Put the section in the fluid and, having warmed a cover-glass, lay it on over it,—not exactly horizontal, but to avoid air bubbles,—lay the edge of the cover-glass upon the slide, let it down

gradually and gently upon the fluid, pressing it horizontal afterwards. If still, there are air bubbles, warm the preparation to fluidity again and gently lift up one edge of the cover-glass a little. They will usually come out; if not, they must be allowed to stay. Several small sections may be mounted under one cover-glass, by carefully distributing them about in the drop. If, in putting on the cover-glass, they should get displaced and overlie each other, warm the slide again, and with a stiff hair thrust in under the cover-glass push the sections into place.

Before putting on the cover-glass, in the first place the preparation should be examined carefully under suitable magnification, and, if any particles of dust or dirt are in the fluid, they should be carefully removed with a needle. For this purpose the simple or compound mounting-microscope is necessary, and we will now direct our attention to learning the use of that.

Let us suppose that the student has the small Zeiss preparing-microscope, or any other of like construction. Over the stage, *ot*, Fig. 12, is an horizontal arm carrying a doublet, *d*. The arm is attached to a steel rod, *st*, which rotates and moves up and down within a sheath, the latter movement giving the coarse adjustment. The fine adjustment is secured by turning the screw, *sr*. The instrument is mounted on a substantial and suitable block whose raised ends, *p*, serve as a support to the hand in manipulating the preparation. The instrument carries two and sometimes three doublets, with a magnifying power respectively of fifteen, thirty and sixty diameters, and may also with advantage be furnished with magnifying glasses having powers of five and ten diameters.

The large preparing-microscope of Zeiss, or another of like construction consists of a lens system Fig. 13, *l*, in which three achromatic lenses are united in one objective,

ob, a tube, and an achromatic concave ocular, *oc*. For low magnification the objective may be used alone as a magnifying glass, by removing the tube and the ocular. The

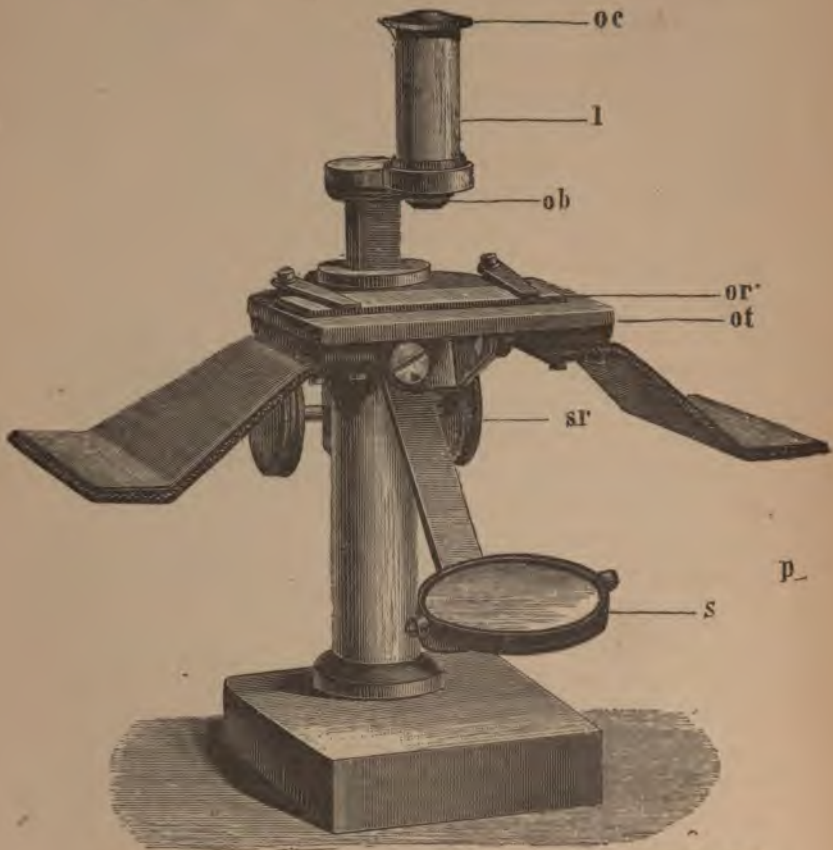


FIG. 13. Larger preparing-microscope of Zeiss, $\frac{1}{2}$ natural size. *st*, stage; *p*, hand-rest; *sr*, rack and pinion; *l*, lens system; *ob*, objective; *oc*, ocular; *or*, an object slide on the stage under the spring clips.

lenses are also separable and we may use the upper alone, or the upper two or all three together; the resulting magnification being fifteen, twenty and thirty diameters, respect-

ively. Focus with the screw, *sr*. On both sides of the stage, *ol*, are rests, *p*, for supporting the hands while at work.

In order to use the compound, as a preparing-microscope, we must attach the erecting prism to ocular No. 2, or replace this ocular by one to which a prism is attached or one may use the erecting ocular, only that these go only with stands having a draw-tube. One can indeed train himself to work with the compound microscope without the erecting apparatus, but it is a matter of much difficulty as all the motions seem to be reversed in the field of the microscope. Hand-rests are also necessary in any case.

Whatever microscope we use, we place the preparation, from which we are to remove the foreign substances, on the stage. After arranging the mirror and focussing the object, we take a needle by the holder in each hand, supporting the hands on the rests, and bring the needle points both at the same time into the field of vision. We shall soon learn how to make the necessarily very small motions required, and shall have removed all foreign substances from our preparation with our needle points. Having again warmed our jelly we put on the cover-glass.

Glycerine jelly preparations require no cementing and are therefore extremely simple to make, and since most vegetable objects and the stains used upon them are well preserved in this medium, it may be commended before all other methods.

Each permanent preparation should be labelled with the name of the plant, the object, the preserving medium, the principal stains used and the date.

We will now go on, and first cut a section, as before directed, of the seed of the white lupine, *Lupinus alba*, or of some related species, moistening the cut surface

with water. Examined in this liquid, the section shows, in the cells, rounded aleuron grains and vacuoles. The grains are strongly refractive, angular, and towards the interior become gradually reticulated and granular. They lie close together and fill the cell, being embedded in a small quantity of fundamental substance, which also lines the cell walls. The latter are thickened and dotted, a structure which we shall study further, with more favorable objects. The grains are colored a beautiful gold yellow with iodide glycerine.

We will next make a section from a *Ricinus* seed. The

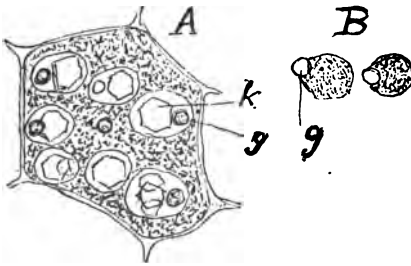


FIG. 14. From endosperm of *Ricinus communis*. *A*, cell with contents under water; *B*, single aleuron grains in olive oil; *g*, globoids; *k*, albumen crystals. $\times 510$.

tissue of the endosperm readily lends itself to section-making as it contains much oil and does not need to be moistened. Examined in water, this liquid expels the oil from the fundamental substance. The grains embedded in this fatty sub-

stance, Fig. 14, *A*, have within them mostly one but sometimes also two or more albumen crystals, and in most, also a single round body which is an inorganic substance (a globoid), a combination of phosphoric acid with lime and magnesia. By the prolonged action of water, the fundamental substance will be disorganized, large drops of oil will collect on and in the object, and on the glass, and on this in irregular masses. But if they float free in the water they are globular. If we focus upon the optical transection of such an oil globule it will appear to be a bright gray and be surrounded with a slender black band.

Raise the tube and the small dark band becomes somewhat broader. Lower the tube and the band disappears, the disk showing itself somewhat more brightly bordered. The drop of oil exhibits an appearance the exact opposite to that which we observed in the air bubble. The air refracts the light less, the oil more powerfully than the water, hence their contrary behavior.

Adding now a drop of absolute alcohol to the *Ricinus* section in water, the preparation is made more clear and the crystals of albumen in the aleuron grains are brought out sharply to view. They become so very distinct that this method of examining them is highly commended. They are crystals of the tetrahedric hemiedrys of the regular system (2). Prolonged action of the alcohol dissolves the *Ricinus* oil drops, it differing from other fatty oils in being miscible with alcohol.

If now we put a section of the *Ricinus* seed in glacial acetic acid the albumen crystals will disappear in the aleuron grains, the latter in their turn increasing considerably in size. The globoids also become larger and show themselves very distinctly in each grain. No drops of oil are seen, *Ricinus* oil mixing with acetic acid, unlike that of other plants. Alcohol and glacial acetic acid, inasmuch as they dissolve the essential but not the fatty oils, make a good test for distinguishing these substances under the microscope.

Turpentine among the essential oils dissolves somewhat less readily in these reagents than do the others. Chloroform and ether dissolve fatty and essential oils in the same manner.

Add a drop of dilute alcana tincture to a section in water and the fatty masses will be colored a red brown. The same result follows with essential oils and resins.

To a glycerine preparation of *Ricinus* seed, add a small

quantity of hæmatoxylin and it will color the albumen crystals a beautiful violet. With olive oil the albumen crystals are not visible. The whole grain appears as a strongly refracting rounded body in one end of which the globoid seems to form a vacuole, Fig 14, *B*. If the section be laid in a one per cent solution of perosmic acid the albumen crystals come out beautifully. They slowly assume a brown shade. Both essential and fatty oils are slowly blackened with perosmic acid.

Albumen crystals of extraordinary beauty, which readily exhibit all the reactions of albumen, are found in the endosperm of the seed of *Bertholletia excelsa*, which may be bought anywhere under the name of Brazil-nut. The section is easily made. Add absolute alcohol to an aqueous preparation and the crystals come out most distinctly. The alcohol does not perceptibly affect the fatty oil. The latter is unchanged by the action of glacial acetic acid, but the albumen crystals soon dissolve. In a one per cent solution of perosmic acid the crystals are very distinct. The crystals are so large that they may be recognized by their form alone, even with comparatively small magnification. Globoids, in the shape of irregular masses of rounded forms, are found lying in the tissue with the crystals. The fundamental substance is very rich in oily matter and gradually becomes almost black with the one per cent osmic acid. The granular contents of the aleuron grains soon turn dark while the crystals are slowly colored yellow. The crystals are optically uniaxial, hexagonal rhombahedra-hemiedrich.

NOTES.

- (1). See Pfeffer, *Jahrb. f. wiss. Bot.*, VIII, p. 429.
- (2). Shimper, *Unters. ü. d. Proteinkrystalle d. Pfl. Inaug. Diss.* Strassburg, 1878.

LESSON III.

STREAMING MOTION IN PROTOPLASM. THE NUCLEUS. DRAWING WITH THE CAMERA. DETERMINING THE MAGNIFICATION.

SELECTING the hairs on the stamens of the *Tradescantia*, as a most favorable object, we will now study the appearances of motion in the living protoplasm. *Tradescantia Virginica* and the species nearest related to it are cultivated in every botanic garden and bloom from May to late autumn. Select hairs from a just-opening or recently-opened blossom. Tear away a tuft of the hairs from the flower with the forceps and transfer it to water. The whole filament may be put under the cover-glass if the anther has been removed. In that case bubbles of air usually get entangled among the hairs which costs much trouble to get out. It may be done, however, by brushing it with a fine pencil while it is still held in place. Put on the cover-glass and if the air has been removed with sufficient care the hairs have not been injured. The hairs will be seen to consist of a series of swollen cask-shaped cells joined end to end, and separated by division walls at the constricted places. Each cell, Fig. 15, shows a thin complete layer of protoplasm lining the cell wall and numerous streams of protoplasm, of various dimensions running through the interior. Suspended within these streams, and enclosed in a coherent layer of plasma, is the nucleus



FIG. 15. Cell from staminate hair of *Tradescantia virginica*. $\times 240$.

(somewhat below the middle in the accompanying illustration). A violet colored cell-sap fills the interior of the cell, covering the nucleus and penetrated by the protoplasm streams. Protoplasm consists of a colorless viscid substance called hyaloplasm which bears numerous minute granules, the microsomata. There are also larger strongly refractive bodies which appear to be of a bluish color and which we will call leucoplasts or starch-builders. If we focus on the protoplasmic wall layer, we shall see that it does not move as a whole, but that fine netlike anastomosing streams run through it. The movement is especially strong in the strings of plasma which penetrate the cell cavity. These streams are of different thicknesses, anastomose laterally quite often, and show a prevailing tendency to meet at the nucleus. Most of the streams end in the plasma layer which surrounds that. The streaming movement is often only in one direction; but often also in a very slender string two streams are seen moving in opposite directions. We perceive the movement by the motion of the microsomes and leucoplasts. Prolonging the observation, we notice that the strings slowly change their thickness, arrangement and configuration. New connecting branches are pushed out, old ones grow thin, snap asunder and are withdrawn into others. So the image constantly changes. The nucleus is almost globular, in many cases oval or somewhat flattened. With the highest magnification which we employ, it appears finely dotted, and in it may also be distinguished some larger grains. In some cells the nucleus seems to have divided itself into two which lie close together. The nucleus is towed about by the plasma strings and so gradually changes its place in the cell. To demonstrate this, make a rough sketch of the cell and contents, and after some little time compare it with the then position of the nucleus and the streams.

To make this sketch at all valuable it should be quite accurate, and hence should be drawn with the camera. Let us learn how this is done.

In Fig. 15½ is given an ideal section of the Abbé camera lucida, which, after focussing the object, should be attached to the ocular and fastened by the screw *sr*. It will perhaps be safest to take the ocular out of the tube for this purpose, and avoid the danger of pushing the objective down upon the preparation by the operation. Then adjust the mirror of the camera as in the illustration in-

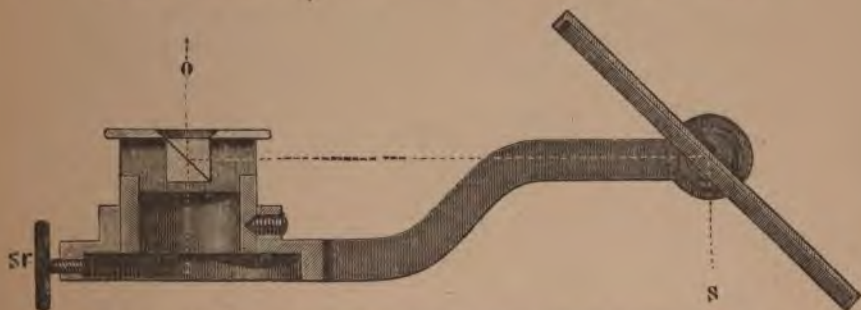


FIG. 15½. Abbé's camera lucida, natural size, longitudinal section. The rays follow direction of the lines. *O*, direction to the eye; *S*, direction to the drawing surface; *sr*, clamping screw.

clining it at an angle of 45° . Now, we shall see an image of surrounding objects in the field of the microscope, when we look into the ocular through the camera. Place a drawing board horizontally, by the side of the microscope under the mirror; on this lay the drawing paper and on this hold a pencil point. If this is visible in the field of the microscope along with the image of the object, the instrument is properly adjusted. The pencil becomes visible by a double reflection, first from the mirror and a second time from the silvered surface of a small prism in the camera (see illustration), while the image of the object comes direct to the eye through a small opening in this silvered

surface. If the drawing board is not in clear view of the observer, the pencil point will be indistinct and the board should be raised up, seldom lowered. The distinctness of the microscopic image on the drawing surface depends upon the relative amount of light on each. That on the drawing surface is regulated by a contrivance of smoked glass attached to the camera.* Having rightly adjusted the instrument and the illumination, trace the outlines of the object in the field with the pencil.

The second camera recommended in the introduction, has the advantage of being always attached to the microscope and ready for use. It consists of two prisms in one mounting, inclined to each other at a certain degree. Rays from the pencil to the eye are brought to be parallel with those from the object, by a double reflection in the prisms. The camera is brought into position when the front edge of the prism, visible through a hole in the mounting, is directly over the eye-lens of the ocular and nearly halves it. It should also be placed close down to the ocular.

The drawing board is inclined, and will be at a proper angle if the circumference of the field of view in the microscope makes a perfect circle when drawn on the board. If it appears as an ellipse when so drawn, the board must be fixed at a greater or less inclination till the field is a perfect circle on the drawing paper as in the microscope.

The same result may be reached by using a stage micrometer ruled to hundredths of a millimeter. Arrange the camera so that the successive rulings will be traced on the board, one beyond the other, using a considerably high magnifying power, and then carefully measure the distance between them. If that distance increase outward the

*The desired balance of illumination may also be obtained by the use of the diaphragm on the microscope, and a small screen which shall throw a shadow upon the drawing board.—A. B. H.

board lies too nearly horizontal; if it diminish it is too nearly perpendicular; if neither, it is just right, and then it will be found to be inclined at an angle of about 25° .

The image which we have thrown down on our drawing board will enable us to determine the magnifying power of our combination of lenses. We know, for example, that the lines are really .01 mm. apart. But on the drawing surface they appear by careful measurement to be 2.4 mm. apart. Hence the magnification equals two hundred and forty diameters.

If one has attained sufficient exactness in drawing to be able to produce a picture of equal dimensions to that of the microscopic image with its given magnification, he has only to measure this and divide the amount by the magnification to get the exact size of the object. This method gives in the simplest way such exact results that we may adopt it exclusively in our investigations. In the example before us the hair cell of the *Tradescantia* measures 9 mm. in breadth. This divided by 240, the magnification, gives .0375 mm. as the real breadth of the cell.*

We will now return and attempt to draw the hair cell of our plant by means of the image thrown down from the camera. We shall have to regulate the illumination of the second camera, by shadowing the drawing board, and properly adjusting the mirror, for we should have an

* What seems to me a far better and more exact way is, to draw all objects which we wish to measure by means of the camera, in either of the following ways:

1. Have the drawing board fixed at a given standard distance of 25 cm. from the camera and so make all drawings at this distance. Then divide the various dimensions of the drawing as above with the known magnification of the lens combination used. To save calculations one may have scales made corresponding to the magnification of the various combinations of lenses by which to measure the drawing at once.

2. Let the drawing board be at any convenient distance from the camera, draw the object, then without changing the relative position of anything replace the object with the stage micrometer and draw the scale of .01 mm. along the edge of the paper. By this the various dimensions are always easily determined.—A. B. H.

equal illumination on the two. Draw with a lead pencil on stiff smooth drawing paper. A very thin solution of gum applied to the finished drawing will prevent it "rubbing" and getting defaced.

We will make a sketch of the outline of the whole, of the streams of plasma and of the nucleus, and after about an hour compare the object and the picture and see if they still coincide with each other. We shall find, as already said, that the divisions of the streams are different and the position of the nucleus has changed.

In order to demonstrate that the streaming motion in the several cells is quite independent in each, and that it is not influenced by the cell walls we will observe the effect on the filament, of an application of a neutral denser fluid like a concentrated solution of sugar, or strong glycerine. Allowing a drop of the fluid to run under the cover-glass and drawing out from the other side a portion of the water with a piece of blotting paper, we shall soon see the effect upon the cells of the hair. The denser fluid absorbs some of the water in the cell, which causes a corresponding contraction of the protoplasmic sac and draws it away from the cell wall at certain points. This contraction of the protoplasmic body under the influence of a water-absorbing fluid is called plasmolysis. While the contraction is not too strong it is observed that the streaming on and from the places withdrawn from the cell wall still continues. Soon, however, all movement in the cell ceases.

It will, however, be resumed again if the denser fluid be replaced by water, and this may be accomplished by drawing out the fluid from under the cover-glass by means of blotting paper, at the same time that water is allowed to run in under from the opposite edge of the glass. The protoplasmic sac will then again be distended till it reaches and rests upon the cell wall. It often happens that when the

protoplasm is withdrawn from the cell wall, little masses of the plasma will be torn away from the cell body and lie as rounded balls on the walls of the cell. All these, however, are taken up and absorbed into the mass of the protoplasm, when it is restored to its normal place and conditions.

One may easily demonstrate that during the above-mentioned contraction of the contents, the coloring matter is not diffused through the living protoplasm, and that the coloring matter of the cell-sap is correspondingly darker. With dead cells the appearance is quite different. The application of absolute alcohol to the hairs immediately kills the protoplasm and causes it to absorb the coloring matter. The color of the cell-sap is immediately withdrawn and it becomes very clear, while the cell plasma and the nucleus are stained a dark violet. The violet coloring matter can now penetrate the protoplasmic sac and distribute itself in the surrounding fluid.

In lack of *Tradescantia*, other plant hairs may be substituted, as, for example, those from the youngest sprouts of the *Cucurbita* species. Cut them at the base from the plant, with a razor, and transfer to a drop of water on the slide. The stouter hairs are composed of several cells at the base, but change into a pointed row of cells upward, while others bear little many-celled knobs on their points. The network of protoplasm is richly developed in the cells and contains microsomes and larger, less numerous, green-colored chlorophyll grains. The nucleus is large, suspended in the threads, has bright nucleoli, and is moved about here and there in the cell.

The root hairs of the *Hydrocharis morsus ranæ* afford a very characteristic object. Take the young, fresh roots with stiff hairs which are visible to the naked eye. Cut off the end of the root and lay it under the largest cover-glass in a sufficient quantity of water. On account of the considerable thickness of the object, all parts of it cannot

be brought within the focus of the stronger magnifications, the lens striking the cover-glass before the deepest parts of the object are in focus. The hair cells are very long, sac-like. All root hairs are single-celled. The rich protoplasm is in powerful motion; but there are no fine, net-like, many-branched streams, only a single, strong, recurrent wall-stream. We distinguish this kind of streaming from the other, the circulation, by naming it "rotation."

This stream is a broad, slightly screw-like, recurrent band which, if projected upon a plane, would form a very elongated figure 8. We may not, perhaps, represent the movement, as if the band as a whole rotated within the cell, since we observe that the neighboring particles continually change their relative position during the movement. The two streams, moving in opposite directions, do not indeed immediately impinge upon each other, but are separated by a thin layer of plasma which remains at rest.

The rotation of the protoplasm is well illustrated in the cells of the leaf of *Vallisneria spiralis*. Make a section from the under side of a stout leaf, by laying the long, slender leaf over the index finger of the left hand, holding down the ends with the thumb and third finger, and then make a superficial section, with a razor, of about half the thickness of the leaf, and lay it on the slide with the cut surface up. Find a place where no attached air bubbles interfere with the observation, and then selecting as wide and long a cell as possible, look for the streaming movement. The movement is retarded by lowering the temperature and, consequently, accelerated by slightly warming the slide. The stream circles about the whole cell without essentially deviating from a direction parallel to its longer axis.

The "indifference layer" has considerable breadth. The stream carries about the nucleus and the green chlorophyll grains. The former is flattened disk-shaped. It is for

the most part hidden by the chlorophyll grains, but occasionally comes in sight. Frequently, it gets stuck fast in some depression or turning place, and then the chlorophyll grains get dammed up against it, till a moment later all get drawn into the stream again. The direction of the movement changes from cell to cell without regularity. By adding glycerine or sugar solution as before, one may easily see the movement continue, in the first moment of the contraction of the protoplasmic sac.

The most powerful plasma stream known in vegetable cells is met with in the *Characeae*. We must use the genus *Nitella*, since the internodes of the genus *Chara* have an opaque outer layer which renders them unserviceable for our purpose. We should select one of the younger internodes, and we shall soon observe that the rotating stratum of protoplasm has a very considerable thickness, and that there is an outer layer in which are embedded the chlorophyll grains. This layer does not move. It is in this case, relatively, quite thick, but is commonly so thin as to escape observation; for, in all the other cases, there was an unmoving protoplasm-layer, the so-called "skin layer." An obliquely lying stripe on the wall of the *Nitella*, easily seen, contains no chlorophyll grains. It corresponds to the "indifference layer" of the protoplasm stream. It repeats here the appearance seen in the root hairs of *Hydrocharis* when the "indifference stripe" of the protoplasmic layer is likewise found extremely reduced. The cells of the internodes of *Chara* have many nuclei, and the protoplasm stream bears many elongated nuclei which, only under the most favorable conditions, are discernible as clear spots. The rounded masses which appear in the stream, in greater or less number, are not to be confounded with these. They have either a smooth surface or are covered with minute spines. Nothing is clearly known of their significance.

LESSON IV.

CHROMATOPHORES. COLORED CELL-SAP.

WE have already several times glanced at the structure and contents of the chlorophyll grains, and will now direct our special attention to these forms. For this purpose, we shall choose a widely distributed moss, distinguished for having very beautiful, large, lens-shaped chlorophyll grains, and whose leaves, constituted of a single layer of cells, are, without further preparation, most favorably adapted to our purposes. This moss is *Funaria hygrometrica*.



FIG. 16. Chlorophyll grains from the leaf of *Funaria hygrometrica*.

Numerous chlorophyll grains of considerable size are seen in each cell; and, in plants growing in diffused daylight, are distributed only on the free cell walls, that is, on the walls which constitute the upper and under surfaces of the leaf. They present their broad side to the observer. That they are smaller when seen in profile we observe in those occasional instances when they are found lying on the side walls of the cells. Every stage in the process of self-division of the chlorophyll grains is easily found, often in one cell. (See Fig. 16.) The resting grains appear almost circular. Then they become elliptical, then biscuit-shaped and finally completely dissevered. The two young grains remain for a long time in contact. The starch contents of the chlorophyll grains are, according to their various sizes, in many leaves easily and in others with difficulty seen, but the starch comes out clearly if the chlorophyll grain is set free in the water and disor-

ganized. For this purpose, cut the leaf into small pieces with sharp shears, and the freed starch from the chlorophyll grains will increase in size in the water and may be easily detected with iodine.

An uninjured chlorophyll grain treated with iodine is colored brown, this being the result of the combined blue coloring of the starch, the yellow brown of the protoplasmic fundamental substance, and the green of the chlorophyll. The better way is to bleach a leaf by long immersion in alcohol. Then the iodine solution, gradually penetrating the colorless chlorophyll grain, will color the starch within before it does the protoplasmic body. The iodine reaction is greatly assisted by the use of potash which swells the starch grains and thus makes the least possible quantity of starch visible in the chlorophyll grains (1). A like result is better obtained in fresh grains by treating them on the slide with a solution of five parts chloralhydrate in two parts water (2) to which a little iodine tincture is added. The chlorophyll is dissolved so that after a few minutes the leaf becomes colorless; at the same time the chlorophyll grains and their starch contents are swollen and in the latter the blue color comes out distinctly. Alcohol-bleached leaves, so treated, behave in the same way. If treated with a very dilute aqueous solution of methyl violet or gentian violet, the cell membranes are stained, but the grains still more and become more distinct.

The living chlorophyll grains of the *Funaria* leaf appear to be finely dotted under a high magnifying power and so betray a reticulated structure.

The same results may be had with the prothallium of the fern so that either object may replace the other. The prothallium may be found in any greenhouse where ferns are cultivated. Any species will do equally well.

For the study of other colored elements (3) we will take an opening blossom of *Tropeolum majus*. In the older blossoms the colored bodies are beginning to disorganize. With the forceps thrust into the tissue, tear off a piece from the upper side of a sepal. Lay the strip in a drop of water on the slide, the epidermis up.

Make the examination at once, before the water spoils the colored bodies, and select an uninjured cell. The



FIG. 17. From the upper side of the calyx of *Tropeolum majus*. Under wall of an epidermal cell with color bodies lying on it. \times 540.

colored bodies are yellow with a shade of orange. They are spindle-shaped three- to four-angled. (See Fig. 17.)

The uninjured ones are homogeneous. Water swells them, rounds them out and makes vacuoles or water-filled spaces, in their interior. These bodies are especially numerous on the inside wall of the epidermal cells of the upper side of the calyx. The brown stripe on the upper side of the sepals arises as a section would show from the red cell-sap which fills the epidermal cells. These cells contain also yellow grains which the colored cell-sap renders almost invisible. The nucleus of the red cells appears mostly as a clear spot. The

petals show corresponding relations. The edges of the lamella, as well as the cilia at their base, may be used for our observation. If attached air bubbles interfere with the examination, a slight pressure on the lamella will drive them away. But the sepals are to be preferred to the petals for examination of the color-bodies, on account of the papilla on the latter, which, by their own form and the quantity of air that they entangle between them,

materially interfere with the observation. The fiery red places, at the base of the petals, arise from the rosy cell-sap and yellow grains in the epidermal cells.

During the examination, it has been observed that the upper surface of the epidermal cells of the upper side of the sepals are longitudinally striped. The stripes pay no regard to the boundaries of the several cells, and are folds of the cuticle covering the epidermis.

The color-bodies are fairly well fixed with iodine water and are, at the same time, colored green and become very distinct. The nucleus is stained a yellow-brown, and its nucleoli are distinctly brought out. With methyl or gentian violet, the color-bodies are stained violet.

Yellow coloring matter is almost always connected with a protoplasmic substance, but we sometimes find it dissolved in the cell-sap, as in *Verbascum nigrum*. Put the petal directly in the drop of water and remove the attached air as much as possible.

In the epidermal cells, on either side of the leaf, which have a wavy outline, the yellow cell-sap is seen. The brown spots are caused by purplish-brown cell-sap. In the epidermis of the stamens, a thin lamella of which may be taken off with the razor, is the yellow cell-sap; also an irregular mass of cinnabar-red coloring matter, and a number of colorless leucoplasts filled with starch.

In the under lip of the corolla, *Antirrhinum majus*, is a sulphur-yellow cell-sap. The red portions have, in their cells, a rosy cell-sap and, partly, also one, seldom more, carmine-red balls of coloring matter.

Blue cell-sap may be found in the epidermis cells of the corolla of *Vinca major* or *minor*. The epidermal layer may be easily torn away with the forceps. The side walls of the cells have ledges projecting into the cell cavity,

Fig. 18, which are swollen at their inner edge, so as to become T-shaped, and on account of the effect of unequal refraction have the appearance of folds.

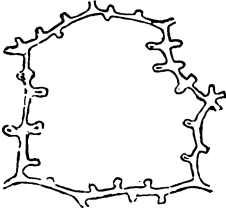


FIG. 18. Epidermal cell of the under side of the petal of *Vinca minor*. \times 540.

Rosy cell-sap gives the color to the rose. The epidermis may be easily torn away. It is deeply papillated and velvety. The cuticle is marked by distinct stripes.

The epidermis of both sides of the blue sepals of *Delphinium consolida* consists of cells with wavy contour. On the upper side the cell rises into a papilla, so that by focussing at about half its height, we get a sun-like figure. The cells contain blue cell-sap, bordering somewhat on the violet. Besides this, many cells contain blue stars, which are produced by short needles crystallized from the coloring matter. The epidermis may be torn away in small pieces. But the sepal is transparent enough to be examined through its whole thickness at the edges, after removing the air.

Examples of red and blue cell-sap may almost always be met with in red and blue flowers, among the more striking of which, is the intense red-colored flower of *Adonis flamens*. Stripping off a piece of the epidermis with the forceps, we see in the cells beautiful red, nearly round to elliptical grains, nearly as large as chlorophyll grains. They appear to be finely granular, and, in water, dissolve into very fine granules which exhibit the molecular motion. The epidermal cells are elongated, the cuticle longitudinally striped, the stripes running distinctly across the boundaries of the cells.

The orange-red color of the root of the *Daucus carota* arises from carmine and orange-red, crystalline, colored

bodies ; their commonest form is represented in Fig. 19. they are small, right-angled rhombs, often elongated to a needle shape, again prismatic and often fan-shaped. To these crystalline forms are often attached small, laterally-projecting, starch grains. These crystals are, therefore, original sources of starch, like chlorophyll grains and other colored bodies ; but the crystallized coloring matter determines the form ; the crystals have but a very small quantity of plasma from which the starch originates.

If we examine the variegated varieties of trees and shrubs, or even of the herbaceous plants which have red-brown leaves, we shall find the cells of the epidermis filled with a rosy cell-sap, and the compound red-brown color is the effect of the red on the surface and the green below.

The red, autumn color of the leaves of the woodbine, *Ampelopsis hederacea*, is caused by the rose-colored cell-sap in the cells of the tissue. Distinct yellow autumn colors of leaves arise from the disorganization of chlorophyll grains, as is most beautifully shown in the leaves of *Gingko biloba*, or, lacking these, of the maple species. The brown color of some leaves comes from the corresponding color of the cell walls, but principally of the cell contents, as may be easily seen in the oak.

Starch grains originate in specially individualized protoplasmic forms, as in chlorophyll grains, also in the color substances, where starch grains may often be detected, and, finally, also in colorless starch generators ; the latter assists in the formation of starch in the deep layers of the plant body. We may name all these together chromato-

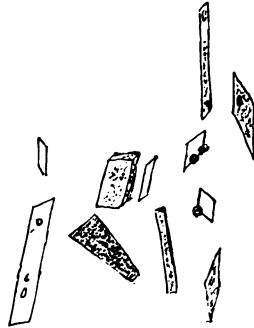


FIG. 19. Color-bodies from the root of carrot. Part with starch grains. $\times 540$.

phores, and, again, the chlorophyll bodies chloroplasts, the colored bodies chromoplasts, and the colorless starch generators leucoplasts. These forms are nearly related and may be transformed into one another; they all belong to the protoplasm of the cell in which they lie embedded.

On the contrary, the blue stars which are found in the cell-sap of *Delphinium consolida* do not belong here, but are a colored substance crystallized from the cell-sap. Likewise the colored lumps which we found in the red cell-sap of *Verbascum* is not a chromatophore.

The largest and most beautiful starch grains are produced in the leucoplasts, and still the leucoplasts are not easily seen; a relatively, favorable object and one not dif-



FIG. 20. Leucoplasts with starch-grains from root of *Iris germanica*. \times 510.

icult to obtain, for this purpose, is the rhizome of *Iris Germanica*. Make a section parallel to the surface of the rhizome; directly, the outer-tissue layer is removed, we come to the starch layer; examine in water. In uninjured cells, the leucoplasts appear as collections of plasma on the posterior end of the starch grains, Fig. 20. The latter grow here only, and, therefore, have an eccentric structure.

The leucoplasts become granular under the eye of the observer, and, finally, dissolve in small grains and show the molecular movement. Two starch grains are often found in one leucoplast; such grains soon touch each other and henceforward have layers in common; these and like causes produce in this and in other cases compound starch grains.

NOTES.

(1) Methode von Böhm, Sitzungsber. d. K. A. d. W. in Wien, Bd. XXII, p. 479.

(2) Nach A. Meyer, das Chlorophyllkorn, p. 28.

(3) A. F. W. Shimper, Bot. Ztg., 1880, Sp. 881; 1881, Sp. 185; 1883, 105 und Sp. 809; A. Meyer, das Chlorophyllkorn, Bot. Ztg., 1883, Sp. 489.

LESSON V.

TISSUE, THICKENING OF THE WALL, REACTION ON SUGAR, INULIN, NITRATES, TANNIN, WOOD SUBSTANCE OR LIGNIN.

FROM a piece of the white sugar-beet cut a section parallel to the longer axis and in the direction of the radius at right angles with the visible rings of the root. Examined in water, it will be seen to consist of nearly right-angled cells filled with a watery colorless fluid. The cell walls are dotted with bright round or oval pits. In occasional cells the nucleus is visible. The intercellular spaces are mostly filled with air. In places the parenchyma cells become slenderer, are elongated lengthwise of the root and between them are tubes, mostly air filled, with peculiar thickening of the walls. These tubes are vessels. Thickened reticulated ledges cover the walls, thin places lying between. These thin places or pits are elongated transversely to the length of the vessel. Ring-like thickenings may be seen now and then projecting from the inside of the vessels. These are the diaphragm-like remnants of originally perfect division walls, and indicate that the vessels originally consisted of a series of cells. The air may be drawn from the vessels by means of an air pump. Lacking this, put the section in recently boiled water, or better still in alcohol. This liquid will kill the cell contents but that does not matter in this case.

Occasionally, we shall meet with a cell filled with small klinorhombic calcium oxalate crystals. The test is that they do not dissolve in acetic acid but do in sulphuric

acid. Make the test with two preparations. The resulting gypsum is so small a quantity that it is dissolved in the surrounding liquid.

Treat the section with an aqueous solution of methyl green or methyl green and acetic acid. The cell wall becomes a beautiful green, and in the latter case the cell contents are fixed and quickly stained. The walls of cells and vessels are colored a bluish green. Not so the pits on the cell walls, which are the thin places on the walls of cells not otherwise much thickened. Every parenchyma cell contains a nucleus, having a distinct nucleolus, and surrounded by minute leucoplast, and a thin layer of protoplasm on the wall. The vessels have neither. To the section in water add chloriodide of zinc and we shall get the characteristic violet cellulose reaction. The coloring begins on the edges of the section, but it may require hours to become complete. The vessels are colored a brownish yellow like lignified membrane. The pits on the cell walls are uncolored and become more distinct. These pitted surfaces are always oval of various sizes irregularly distributed singly or in groups. The larger pitted places are overspread with violet bands of different breadths, making the appearance of fan-shaped irregular lattice work. Bright granules colored yellow brown by the chloriodide of zinc adhere to the pitted surface. For comparison produce the iodine and sulphuric acid cellulose reaction. Impregnate the section with potassium iodide and transfer to dilute sulphuric acid (two parts acid and one water). It will be colored a beautiful blue. The smaller pitted surfaces are still uncolored, the larger ones latticed blue.

Make a section of a ripe pear. The pulp consists of regular thin-walled large parenchyma cells somewhat rounded at the corners, having colorless cell-sap, a much reduced

plasma sac and a nucleus. Scattered in the tissue are nests of strongly thickened cells, Fig. 21. The number of these united stone cells is different in different places and in different species. They form the so-called "stones" of the pear. The cells are distinguished by the considerable thickening of their walls and by the numerous, fine branched canals penetrating the walls. The branching is caused by a number of the canals uniting inwardly as the cell cavity becomes narrower, forming a common canal which opens into the cell cavity. When two thickened cells touch, the canals meet. In the present condition, these cells have no living contents, only a watery fluid. Consequently they represent only dead cell husks. Chloriodide of zinc slowly colors the parenchyma cells violet and the thickened cells a yellow brown. The latter are therefore lignified and on account of their thick-

ness and lignification are called sclerenchyma cells. The structure of thickened cells is well brought out with chloriodide of zinc.

We will use the pulp of the pear for our microscopical study of the sugar reaction. The most common is that with Fehling's solution (34.64 g. pure crystallized copper sulphate, 200 g. tartrate of potash and soda dissolved in water). This solution may be kept on hand. Take about

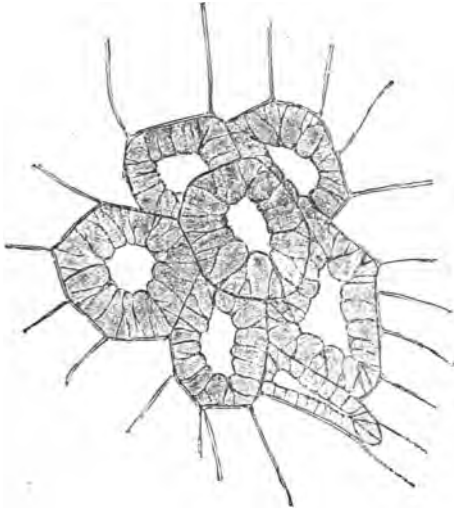


FIG. 21. From the pulp of the pear. Much thickened cells, with branching pore canals. Surrounded by thin-walled parenchyma cells. $\times 240$.

600 ccm. soda lye, sp. gr. 1.12, dilute to 1000 ccm. and boil. The section, which should be not less than two or three layers of cells in thickness, should be transferred from the Fehling's solution to the hot lye, and the reaction at once takes place. The microscope shows in the cells the vermilion-red precipitate of reduced cuprous oxide. There is therefore in the cells of the pear a substance which will reduce an alkaline cupric oxide solution, a substance belonging to the grape sugar group (glucose), in this case grape sugar.

For comparison, make the investigation with a section of the sugar beet; this contains, as we know, cane sugar. Immersing the section, for a couple of seconds in the boiling liquid, gives no precipitate in the cells; the section becomes blue; if the section lies for a long time in Fehling's solution, the surface begins to show the vermilion-red color. The cane sugar has undergone transformation and now gives the cuprous oxide precipitate. Under the microscope, vermilion-red granules appear on the peripheral cell layer, while, if the reaction has not continued too long, the inner cells contain a blue liquid.

For microscopical purposes, the Barfoed sugar reaction with acidulated copper acetate has much to commend it. Dissolve one part neutral, crystallized copper acetate in fifteen parts water; to 200 ccm. of this solution add 5 ccm. acetic acid, which contains thirty-eight per cent glacial acetic acid; in a test-tube, holding from 5 to 8 ccm. of this solution, put a not too thin section of the pear and in another a section of the sugar beet, and boil up for a short time; pour all out into small glass dishes and let them stand. After some hours we shall find the pear section covered with a fine precipitate of cuprous oxide and a small quantity also of the precipitate in the bottom of the dish, while the beet-root section has none of it. The effects of the reaction should be compared after a few hours,

since a small quantity of the precipitate is reoxidized in the air after a longer time and may then be dissolved.

We, finally, use the sugar beet to observe the nitrate and nitrite reaction by means of diphenylamin (3). This substance, used by the chemist as a most delicate test for nitrates and nitrites, is very useful for histological research. Make any section of the beet which shall reach the outer surface, lay it on the slide, partly dry it and add the reagent; this consists of 0.05 g. diphenylamin, in 10 ccm. pure sulphuric acid. Immediately, there appears in the outer zone of the section, an intense blue color; this zone contains

the latest product in the developing tissue of the beet, and, consequently, is that part which contains the nitrate. Directly, the blue color begins to spread over the rest of the section, but, at first, the reaction in the colored zone is quite sharply defined. We conclude that it is a nitrate and not a nitrite,

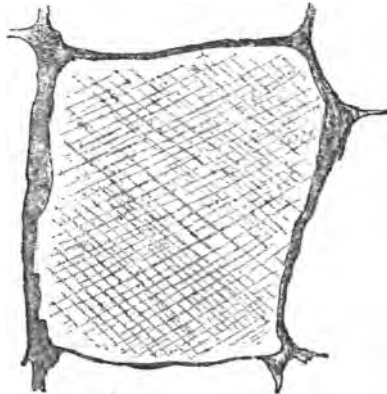


FIG. 22. From the pith of *Dahlia variabilis*.
× 420.

which we find here, because the former is much oftener found in the analysis of the juices of the plant. We partly dry the section, so that the reagent and the color will not spread so rapidly over it, and the colored zone will be more sharply defined.

Take next the dahlia bulb, *Dahlia variabilis*. The longitudinally-halved bulb shows the central pith; a longitudinal section of this shows, under the microscope, many series of nearly rectangular cells, Fig. 22, with a

much reduced protoplasmic sac with nucleus and cell-sap, the intercellular spaces being filled with air, and the cell walls finely striated, the striæ lying at an angle of about 35° to 40° . There seem to be two opposite systems of striæ on each wall; but, in fact, they belong to two adjacent walls, and the extreme tenuity of these walls enables us to see the two systems at once. The cell walls are

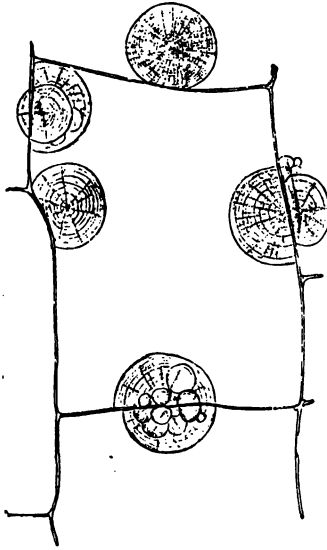


FIG. 23. From the bulb of *Dahlia variabilis* after lying in alcohol several months. Sphere-crystals on the walls. $\times 240$.

colored violet with chloriodide of zinc, but when the stripes do not approach each other very closely, a colorless line is seen between them. Like the pitted surfaces of the wall, these unthickened places are not colored by the chloriodide of zinc. Single, relatively large, rhombic-shaped places come out with great distinctness as pits; these pits always lie on the dividing line between two striæ and at the places where the dividing lines of one system cross those of the other.

Put a section in absolute alcohol and a fine precipitate of inulin will be produced in the cell-sap. Replace the alcohol with water and warm the slide, and the precipitate will be again dissolved. In order to study the spherical crystals which the inulin forms, one should take a piece of the bulb which has been in alcohol at least eight days; examine the section in water and add nitric acid very slowly at the same time. The spherical crystals, Fig. 23, are always found on the cell walls; they

form more or less perfect globules which may be broken through by one or more cell walls. For the most part, globules of different sizes form together a large group; the globules exhibit more or less clearly a radial structure, which becomes more distinct after the nitric acid begins to affect them; this arises from the radially arranged crystal needles, of which the spherule is built. Besides the radial structure, a concentric lamination is visible which is understood to signify an unsteadiness in the conditions of crystallization. Iodine solutions do not color these spherules. Warmed in a drop of water on the slide they soon disappear.

There is nothing better than a gall apple for testing the tannin reaction. The gall apple is found on the leaves of the oak and is produced by the sting of the gall wasp, which thus lays an egg in the tissue of the leaf. Halve the green apple and make a delicate radial section. The cavity occupied by the larva is surrounded by a shell which is formed of isodiametric oval cells. These are mostly richly laden with starch grains. The tissue which incloses this consists of radially elongated, polygonal cells, which diminish in length toward the periphery of the apple, and finally end among the small cells, towards the outside strongly thickened, outermost cell layer of the epidermis. There is no definitely formed cell-contents in all this tissue which surrounds the inner shell. But lay a freshly prepared section in an aqueous solution of ferric chloride or ferric sulphate, and the whole mass will be colored a deep blue. This color is imparted to the surrounding liquid and gives us the iron-blue reaction of tannin, which may also have an iron-green reaction. Watching the reaction under the microscope, by putting a dry section under the cover-glass, and then adding a drop of the iron solution, we see that at first a dark blue precipitate is thrown

down, which soon dissolves in the reagent and fills the cell with the blue fluid. The starch-filled cells of the inner shell give the weakest tannin reaction. For comparison we will lay a second section in an aqueous ten per cent solution of potassium bichromate, and we shall see a thick flocculent, reddish-brown, permanent precipitate form in the cells. We shall not examine the vascular bundles of the gall apple since they have nothing peculiar to do with the tannin reaction.

If the stem of a stout *Vinca major* be cut off near the ground and then broken across, we shall see numerous small fibres projecting from the edges of the broken parts. Seizing them with the forceps, we pull them out and put them on a slide in a drop of water. We shall find them to be long, pointed, much thickened sclerenchyma fibres. The cell cavity is reduced to a narrow tube, and towards the ends quite obliterated. In the less thickened walls we find but one, and in the more thickened, two systems of striation, the one belonging to the inner and the other to the outer layer. In very old sclerenchyma fibres, often a third inner system is seen almost perpendicular to the longer axis of the fibre. The latter is derived from reticulated thickenings, the elongated dots appearing between. These innermost thickening systems are most sharply separated from the outer. With chloriodide of zinc the fibres are colored a violet bordering on the brown. Especially instructive is the behavior of cuprammonia which dissolves pure cellulose. The effect must be quickly and closely watched. The reagent greatly swells the walls of the fibres, at first making the striation more distinct, but quickly obliterating it. The outer layer is soon perfectly dissolved while the inner reticulated structure longer withstands the action of the reagent, and consequently becomes at last fully isolated. At the beginning of the swelling, a still

finer lamination shows itself in the already visible layers. So each layer is composed of a number of extraordinarily fine lamellæ. This very fine lamination is especially well seen in the inner resisting layer.

Split a seed of the Star-of-Bethlehem, *Ornithogalum umbellatum*, with a pocket knife and make a very thin section, with razor, hand-vice and drop of water. The preparation will show nearly right angular cells as in Fig. 24. The walls are much thickened but they are perforated by a number of simple pits. Looking upon the surface of the wall, these pits resemble round pores, *m*. From the side they resemble canals, running from the cell cavity to the primary cell wall. The pits of neighboring walls meet and are separated only by the primary cell wall, *p*, which we call here, the closing membrane. The inner surface of the thickening layer is distinguished by its stronger refraction, and forms the boundary membrane. Add sulphuric acid at the edge of the cover-glass, and the thickened layer will be dissolved,

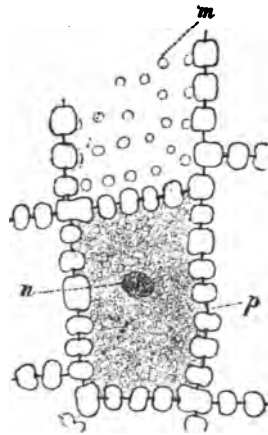


FIG. 24. From the endosperm of *Ornithogalum umbellatum*. *m*, pit from above; *p*, closing membrane; *n*, nucleus. $\times 240$.

while a network of very delicate walls will remain. These walls are the so-called middle-lamella, which correspond to the original walls of the cell before the beginning of the existing thickening, and they also penetrate the closing membrane of the pits. By the long continued action of the acid they too would disappear. Chloriodide of zinc swells the thickened layer and so makes the middle lamella visible. The coloring of the preparation is imperfect in consequence of the swelling.

The cells are closely packed with protoplasm and granular matter, and the whole contents are colored yellow-brown with iodine solution. The nucleus in every cell may be easily demonstrated by means of acetic methyl green. It generally fails in no living cell or a cell capable of life.

The thickened layer of the cells in the endosperm of the date, *Phoenix dactylifera*, has a similar appearance. But the cells are elongated, their cell cavity narrower and their walls thicker. These cells are in the date germ radially arranged. Transverse and radial-longitudinal sections show the cells in longitudinal section, while tangential sections, perpendicular to the radius, show the cells in transverse section. Chloriodide of zinc colors the thickening layer a very beautiful violet, and a prolonged swelling causes numerous lamellæ to appear.

We will now turn to the coniferous wood to study the so-called "bordered pits." Take a piece of an old stem, a dry or alcoholic specimen, and with a sharp knife prepare to make the different sections, a radial parallel to the longer axis, a tangential also longitudinal, and one transverse to this. The concentric annual rings of the wood will give us the necessary points for getting the desired directions. The radial-longitudinal section is cut perpendicular to these rings, the tangential as nearly as possible parallel to them, the transverse perpendicular to both the others. To make good wood sections and not spoil the razor requires the exercise of the greatest caution. In case the razor is ground concave, a section can be made only on the edges of the wood or only so far as that the back of the razor will not strike the cutting surface. Still, razors used to cut wood should be but a little concave, else they will spring and cut unevenly. The best form is that which is ground flat on one side, but this has the fault of not being easily sharpened. The cutting surface should be kept moist, the sec-

tion made as thin as possible. It need not be very large. If the cutting is too deep, withdraw the knife and so run no risk of nicking it. The razor should be very sharp so as not to mutilate the cell-membrane, and separate the inner thickened layer from the outer. An alcohol specimen cuts more easily than the dry wood, particularly if it be subsequently soaked in like parts of glycerine and alcohol. The first section cut by the razor should not be used, as the cell membranes of one side have been mutilated by the pocket knife.

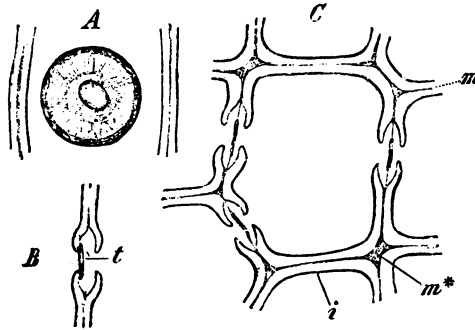


FIG. 25. *Pinus sylvestris*. *A*, bordered pit, side view; *B*, same in tangential longitudinal section; *t*, torus; *C*, transection a whole tracheid; *m*, middle lamella; *m**, a gusset in the same; *i*, inner cell membrane. $\times 540$.

With a low power, a radial section is seen to be built of longitudinally elongated cells pointed at the ends and attached to each other. Crossing over these cells run the cell rows of the medullary rays which we will not now consider. We focus a high power system on one of the broader walls of the longitudinally elongated wood cells and direct our whole attention to the bordered pits of this wall. The bordered pit appears to us in the form of two concentric circles, Fig. 25, *A*. The inner small circle or the inner ellipse represents only the opening of the pit into the cell cavity. The larger outer circle is the

widest part of the pit at which it joins the primary wall separating the two cells. This bordered pit is in fact distinguished from the simple pit as we have seen it in the Star-of-Bethlehem and in the date, only that it widens at its base. The pits of adjacent cells meet in this as in the other cases. If the opening of the pit (as in *A*) is an obliquely placed ellipse, we shall also find by changing the focus that the corresponding pit has its opening inclined in the opposite direction. The two opposite pit cavities are separated from each other by the primary wall which existed before the secondary thickening had begun and was then very thin. This delicate wall is the closing membrane.

This is thickened in the middle and forms the so-called "torus." By careful attention and focussing, this torus may be seen. It forms a smooth, bright, round disk of about double the diameter of the orifice of the pit (see in *A*). In favorable cases and particularly in preparations from dry wood this thickening of the membrane is seen to possess a radial striation; so that it would seem to be differentiated into radially running lamellæ (6).

We shall get a full view of the structure of the bordered pit only by means of a tangential section, since the bordered pits are on the radial walls (7) of the wood cells and will be cut across only by a tangential section. See Fig. 25, *B*.

Look for the pits on the division walls of the widest wood cells, and be not led into error by the sections of the cells of the medullary rays. The image of the dissected pit will be seen only in the thinnest and most delicate part of the section. There they will appear in the form of two open pincer jaws, as in the illustration given in Fig. 25, *B*. Recognizing the structure of this larger pit, other smaller ones will be easily made out. The thicker

the wall the longer the canal and the wider the pit cavity. In the most favorable cases one may see the closing membrane within the pit, with its thickened centre *t*. In the larger bordered pits it is mostly pressed over to one side of the pit cavity and seems to serve the purpose of a valve. The image becomes clearer after treating the section to chloriodide of zinc which colors the cell wall a yellow-brown. Some inner layers not yet fully lignified show a violet flush. The closing membrane is not colored. This reagent demonstrates that this wood cell has neither nucleus nor protoplasmic sac. It consists only of dead cell walls and resembles the vessels in its function of conducting water as well as in the manner of its wall thickening. It is called a tracheïde or more recently a hydroïde.

Often the coniferous wood which we are examining will be seen to have a spiral striation with an ascent of about 45° . The pit openings appear thus to be elongated in the direction of the striæ of the two opposite sides of the wall on which they are placed and so the opposite pit openings seem to cross each other.

We will now make a very delicate transverse section of the wood. The tracheïdes are prevailingly at right angles and mostly arranged in radial rows. On the radial walls of a wide cell we find the pit cut in section Fig. 25, *C*, whose form is not different from that shown in the tangential section. Between the cells is a fine dividing line *m*, the middle lamella. When more than two cells meet the middle lamella is widened into a solid or hollow gusset *m**. The inner border of the cell wall is more refractive and forms the boundary membrane *i*; that of the thick walled tracheïdes is especially distinct. It all becomes still clearer by the use of concentrated sulphuric acid. The thickened layer swells and finally dissolves; the boundary membrane, withstanding its action longest, comes out

sharply to view. Between the swelling, thickening layers are the primary walls of the cells and these at last remain a delicate network colored a yellow-brown. This acid-resisting, middle lamella is "cutinized." By the gradual swelling of the thickening layer in sulphuric acid we find that it is composed of numerous extremely delicate lamellæ. Chloriodide of zinc colors the section yellow-brown but in some cases the innermost part of the thickening layer takes a violet tinge. If we follow the chloriodide of zinc treatment with dilute sulphuric acid (two parts acid and one of water) the whole thickening layer will be colored blue. Treat the section with chromic acid and the middle lamella will be dissolved and the cells separated. The thickening layer will swell somewhat, the lining membrane at first showing up sharply and afterwards disappearing.

Phloroglucin and aniline sulphate give characteristic reactions with wood substances or lignin (8). Dissolve a small portion of the phloroglucin in alcohol and lay the section in the solution. Afterwards put it in a drop of water on the slide and add a little hydrochloric acid at the edge of the cover-glass. Directly the cell walls will be stained a beautiful violet red color. An aqueous solution of aniline sulphate colors wood a bright yellow, but the color is heightened by adding a dilute sulphuric acid. In place of the phloroglucin, one may use an aqueous or alcoholic extract of cherry wood, with almost the same results (9). Treat a section from a fresh stem of fir wood which has either the pith cells or the bark cells, with concentrated hydrochloric acid. Immediately the wood will be colored yellow, which afterwards gradually softens to a violet (10). This is also a phloroglucin reaction, the phloroglucin being derived from the pith or bark cells. The medullary rays themselves in young wood contain some phloroglucin.

Thus the different behavior of lignified and unlignified cell walls toward coloring matter is one important element in their investigation.

NOTES.

- (1) Compare Sachs, finally Jahrb. f. wiss. Bot., Bd. III, p. 187.
- (2) Barroed de organiske Stoffers qualitative analyse Kjöbenhavn, 1878, pp. 210, 217, 223 Anm.
- (3) See H. Molisch: Ber. d. deut. bot. Gesell. I Jahrg. p. 150.
- (4) Sachs, Bot. Zeitg., 1864, p. 77; Hansen, Arb. d. Bot. Inst. in Wurzburg, Bd. III, p. 108; Meyer, Bot. Ztg. 1883, Sp. 334.
- (5) Sanio, Jahrb. f. wiss. Bot. Bd. IX, p. 50; Strasburger, Zellhäute, p. 38; Russow, Bot. Centralbl. Bd. XIII, No. 1-5. There is also other literature.
- (6) See Russow, Bot. Centralbl., 1883, Bd. XIII, No. 1-5.
- (7) Tangentially placed, bordered pits, which are so rare in the fir, quite regularly occur in those wood cells of other *Abietinæ*, which are formed in the autumn.
- (8) Both introduced by Wiesner (See Stzber. d. Math. nat. Kl. d. Akad. d. Wiss. Bd. LXXVII, 1 Abth. und früher schon a. a. O.)
- (9) v. Höhnel, Sitzber. d. Math. n. Kl. d. Wiener, Akad. d. Wiss. Bd. LXXVI, p. 685.
- (10) The same, p. 676.

LESSON VI.

EPIDERMIS. STOMATA.

PREPARE a superficial section from the outside (underside) of the "riding" leaf of *Iris florentina*. The section should be so thin that but traces of the underlying tissue should adhere to the epidermis; examine in water, the outside uppermost. The epidermis consists of much elongated cells running parallel to the axis of the leaf. The cells end in a transverse division wall, are joined together without intercellular spaces, contain colorless cell-sap, a reduced plasma sac and a nucleus. The outside of the epidermis is covered with an extraordinarily, fine-grained wax. On a line with the epidermal cells lie the elliptical stomata indistinctly seen. The four contiguous surface cells reach over and partly cover the guard cells of the stoma, so there remains only an elongated, elliptical, minute cavity, *f*, which leads to the stoma, Fig. 26, *A*; this cavity is filled with air and appears mostly black. Turn the section over now, and it will easily be seen that the stoma is formed of two semilunar shaped cells; unlike the neighboring cells of the cuticle, they contain chlorophyll grains. The nucleus is seen as a clear spot, usually for half the length of the cell. Between the two guard-cells occurs a spindle-shaped opening, *s*, which extends along half the length of the cells. Make a section now, crosswise of the leaf, and you will naturally make it transversely across the stomata. For this purpose, cut out a small piece from the leaf and make the section, while holding it between the two halves of an elder pith. A

piece of the pith, 3 cm. long, may be carefully split in two, lengthwise, the piece of leaf laid between the two halves in such a way as to bring the edge to be cut just above the end of the pith; hold the pith in the fingers or put a light rubber band around it to hold it in place, or even fasten it in the hand-vice, but hold it so that the knife will cut along the broad surface of the object and not

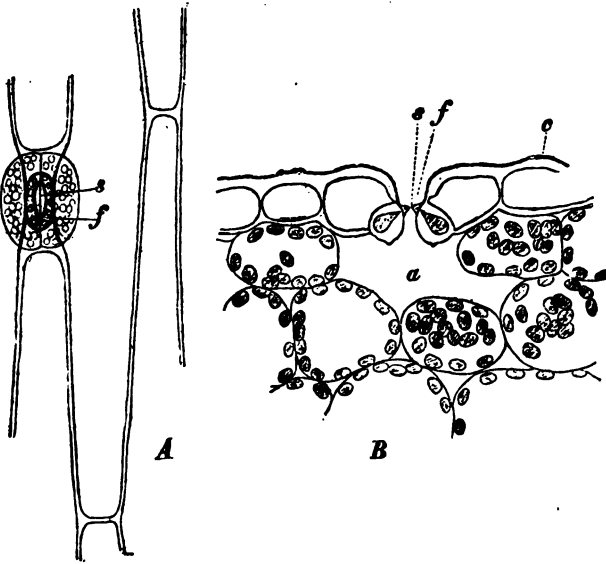


FIG. 26. Epidermis of the under side of the leaf of *Iris florentina*. *A*, surface view; *B*, transection; *f*, minute depression; *s*, stoma; *c*, cuticle; *a*, breathing cavity. $\times 240$.

upon the edge. For very delicate objects, the sunflower pith is better than elder pith. Cut first both the end of the pith and of the object, quite clean away, then cutting through both at the same time, make several very thin sections, keeping the cutting surface moistened and a drop of water on the razor blade. Remove the sections from the knife to a slide by means of a hair pencil. The prep-

aration of sufficiently delicate sections need occasion no great difficulty, but if such a difficulty arise, it may be met with a microtome; a hand microtome of the simplest construction will be sufficient. The pith holding the object may be fitted into a cork and this into the well of the microtome, the pith rising some little above the cork. The section may be made with a common or a flat-sided razor, cutting free-hand across the top on the brass or glass plate, and lifting the object each time the required distances by means of the screw. More elaborate microtomes, useful to the zoölogist, are superfluous to the botanist.*

Make a number of sections and put them in a watch glass. Examining a section in water, we find a stoma cut through the middle, as in Fig. 26, *B*. First, we notice that the cells of the cuticle are thicker-walled without than within. Still the inner walls are pretty thick, while the radial walls are very thin; these are connected with the function of the cuticle which is not only to protect the leaf without, but also to be a water reservoir (2). The thin radial walls are adapted to an easy change in the volume of the cells, for, acting like a bellows, they diminish their height with the loss of water, and, again, increase it when the water is increased. The guard-cells lie deep beneath and between the cells of the cuticle. The little pit, *f*, leads down to the guard-cells; these are much thickened above and below. These thickened sides jut out towards each other in the opening; above these thickened places are peculiar, beak-shaped projections. On the opposite side towards the inside of the cells of the cuticle, the walls of the guard-cells are relatively quite thin. On this method of wall thickening depends the

* Taylor's freezing microtome is very serviceable for cutting soft tissue containing water.—A. B. H.

mechanism for the movement of the guard-cells which are more curved and the orifice opened, the greater their turgidity; and, conversely, are more extended and the orifice closed or narrowed by a decrease of their turgidity. It is, in fact, clear that the guard-cells, by increased turgidity, become more convex on the side of least resistance and concave on the side of most resistance, like a rubber bag with a thicker wall on one side than on the other. If water or air be forced into it under high pressure, the side making the strongest resistance becomes concave, while that making the least becomes more convex. The thin place on the cleft side, where the two thickened ridges jut together, facilitates the flattening of the cells, while they curve out on this side. Therefore, lest the movement of the guard-cells be interfered with, it is joined to the epidermal wall on a suddenly, narrowed side and is fastened to it after the manner of a hinge. Under the stoma is the breathing cavity, *a*, of the same nature as the larger intercellular spaces filled with air, which is bordered with cells containing chlorophyll and is connected with the open cavities found between these cells. Testing with chloriodide of zinc shows us that the walls of the epidermal cells are colored a yellow-brown, through their whole circumference, with the exception of a thin, somewhat wrinkled membrane, and are the so-called cuticle, *c*; this cuticle swells outward at the stoma, forming the above mentioned beak-like projection, which stains yellow-brown with the chloriodide of zinc, and so appears to be cuticularized. As an extremely delicate membrane, the cuticle extends through the stoma over the guard-cells quite to the beginning of the parenchyma cells containing the chlorophyll. The guard-cells are violet in their whole extent. By the application of concentrated sulphuric acid, the whole section is dissolved except

the cuticle including the cuticularized projection of the stoma.

An unusually favorable object for studying the stomata apparatus is found in the *Tradescantia virginica*. The epidermis is formed of polygonal cells mostly somewhat extended in the direction of the leaf. With these alternate narrow stripes of slenderer and longer cells. These stripes appear green on the under side of the leaf, the others gray. The lateral walls of the epidermal cells are furnished with pores, the outer surface is faintly striated. The number of

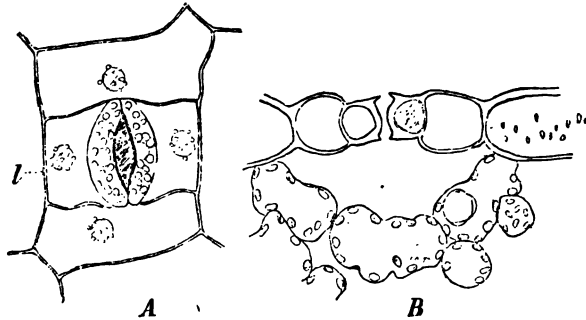


FIG. 27. Epidermis of the underside of the leaf of *Tradescantia virginica*. *A*, surface view; *B*, transection; *l*, leucoplasts. $\times 240$.

stomata is considerably greater on the under than on the upper side of the leaf, so we will examine that side. The stomata are almost always surrounded by four cells, Fig. 27. The guard-cells lie at the same height as the epidermis. The cleft which lies between is relatively large. They have chlorophyll grains between which, for the most part, the nucleus is visible; also in the epidermal cells the nucleus is sharply distinct, surrounded with colorless leucoplasts, *l*, Fig. 27, *A*. The cell-sap of the epidermal cells is here and there rose-colored. Make a cross section,

as with the last example, and we shall have the stoma as in Fig. 27, *B*.

The cleft side of the cells has thicker walls; the other, thinner. The walls of the next adjoining cells are thinner than the succeeding cells of the epidermis, and are the secondary cells of the stomata apparatus, forming the hinge, otherwise provided for in the Iris, as we have seen. The leucoplasts, *l*, surrounding the nucleus are very favorably situated for examination. It is interesting to note that though the leucoplasts are here exposed to the action of the strongest light they remain small and colorless and do not change to chlorophyll grains. *Tradescantia zebrina* has a similarly constructed stomata apparatus, found on the under side alone. The cross section is very instructive even if not very easily made thin enough. The epidermal cells of both sides are of considerable size, particularly those of the upper side, which are so high that they alone make nearly half the thickness of the leaf. Many of them are divided by transverse walls. Mostly the epidermal cells have only watery cell-sap, which on the under side of the leaf is colored red. The epidermal cells of this plant constitute a water-holder of great capacity. The four secondary cells of the stomata are quite flat so that a large breathing cavity of the height of the epidermal cells exists under the stomata. Taking a section from the under surface of the leaf, we can focus down through it and get a good image of it while no air gets into it. The leucoplasts about the nucleus are distinctly seen in the epidermal cells.

The *Aloe* and *Agave* species show an extraordinary development of the outer wall of the epidermal cells and a corresponding depression of the stomata deep in the epidermis.

Take the *Aloë nigricans* found in greenhouses; other

species of the genus may be used in lack of this. A superficial section shows the epidermal cells of both sides of the leaf to be regularly polygonal—mostly hexagonal. The cell cavity is a relatively small, oval space; it is usually filled with air and is black in the section. The stomata occur on both sides of the leaf at the bottom of the deep clefts which are surrounded by four cells and are rectangular and enclosed in a somewhat projecting rim. In order to see the guard-cells, the section must be laid on the slide inside up; these cells are relatively broad and

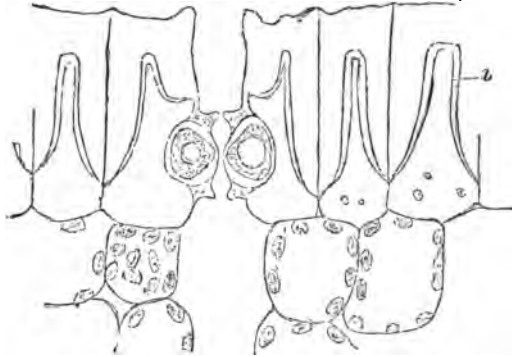


FIG. 28. Transection through the epidermis and stoma of *Aloë nigricans*. *z*, inner thickening layer. \times 240.

short and contain, among other things, strongly refractive, globular oil-drops. The epidermis being so hard, we may use cork in making the section; make the section not the whole thickness of the leaf, but from one side only, cutting from the soft, inner part of the leaf toward the outer and harder, making the section perpendicular to the axis of the leaf. The very great thickening of the cells of the epidermis is at once seen in this section, Fig. 28. The thickening appertains exclusively to the outer wall of the cell. The cell cavity runs out to a point in the same

direction; this thickened wall is white, strongly refractive and is overspread with a cuticular membrane, still more refractive, but not sharply distinct. The lateral boundaries of the cells are marked only by a delicate line indicated on the outside by a slight elevation; the inside of this thick wall is covered by a relatively thinner, less refractive layer, *i*, which surrounds the cone-shaped extension of the cell cavity, and, thinning out wedge-like, ceases at the same time with the thickened layer on the lateral wall. The thickened part of the epidermis in this section looks like a notched curtain. Of the cavity which leads down to the stoma we note first, the projection or rim which encloses it, and also that the tooth which forms the thickened layer is here divided on one side and loses half its height. The guard-cells have a ridge-like, and, in section, beak-shaped projection, both above and on the cleft side; above the guard-cells is a thin place in the wall which forms a membranous hinge or joint. The breathing cavity is deep and narrow.

Parallel striation, more or less oblique, may often be seen on the thickened wall; it is caused by the knife in cutting and often recurs in hard, elastic substances in the same manner. With chloriodide of zinc, the thick wall is colored yellow-brown, showing it to be cuticularized. The inner layer, *i*, and the rest of the leaf tissue, are colored violet. The yellow-brown color extends through the hinge to the two projections on the guard-cells; the remaining part of these cells is tinged violet. Sulphuric acid dissolves all that the last reagent has not colored yellow-brown, and this it dissolves in an hour, leaving only the delicate cuticle and the fine, middle lamella occurring between the epidermal cells. The cuticle extends over the guard-cells to the point where they join the inner cells containing chlorophyll. The cuticle and the epider-

mal layer are colored brown by the acid. The oil in the guard-cells gathers together in a strongly refractive ball on the entrance of the acid and after some time disappears.

In the arrangement of the stomata within the epidermis many modifications occur. A very remarkable one is that when the stomata are surrounded by a single ring-shaped, epidermal cell. The fern *Aneimia fraxinifolia*, found in every botanic garden, will show this. The cells of the epidermis have an extremely wavy outline, Fig. 29, and gain stability by this dovetailing of the edges, so common in epidermal cells.

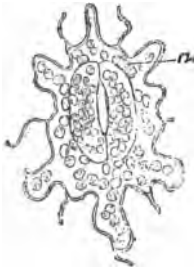


FIG. 29. *Aneimia fraxinifolia*. Stoma surrounded by epidermal cells. *n*, nucleus of epidermal cells. $\times 240$.

In the ferns, the epidermal cells are richly furnished with chlorophyll grains. The epidermis, therefore, belongs with the organs of assimilation as it does not in most phanerogams. The stoma is set in the surrounding epidermal cells as in a rim. A transverse section shows it to be raised somewhat above the surface of the epidermis; this extreme case is connected by transitional forms with others, not treated here, much less remarkable. We should accustom ourselves to think of the stoma, as, in fact, only inserted on the side walls of the surrounding epidermal cells; then the singularity of its insertion will cease.

Nerium oleander shows a peculiar form. Stomata will not, at first, be found either on the upper or under side, but on both sides a uniformly small-celled epidermis, which, particularly on the under side, is covered with single-celled hairs, the walls so thickened as almost to obliterate the cell cavity. On the under side of the leaf

are found certain cavities filled with air, and having on their edges these before-mentioned short hairs; the hairs interlock across the opening and so close up the cavity in front. A second superficial section, taken from the same place whence the epidermis has already been removed, will enable us to get a look here and there into the depth of these cavities. Perhaps an air-pump will be necessary to remove the air, or else an immersion of the section in alcohol; there will be seen projecting from the walls of this cavity small, conical elevations, the apex of which will be formed of a stoma. The lateral walls of these small cones consist of epidermal cells which have a breathing cavity between them extending to the stoma. The same kind of hairs which we saw on the edge of this cavity spring from its walls between the cones.

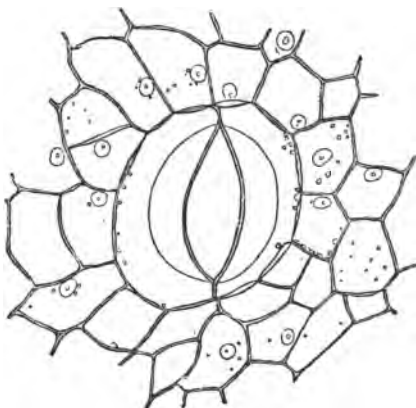


FIG. 30. Water stoma and surrounding epidermal cells from the edge of leaf of *Tropæolum majus*. $\times 240$.

We will now glance at the water pore or water stoma. It exhibits a structure like that of the air stoma, only it is larger, the cleft, together with the intercellular space, at times at least, being filled with water; the guard-cells of this water pore may be unmovable from the beginning, quickly die and then in all cases lose their movability. The best object for studying these structures is the *Tropæolum majus*. The water pore is found on the upper side of the leaf, and, indeed, on the ends of the principal

nerves; here, the edge of the leaf commonly exhibits a small depression. One may approximately see the water pore by putting a piece of the leaf, full thickness in water, under a cover-glass, and place it under the microscope; but the peculiarities of it will really be recognized only when a surface section be made of this part of the leaf; it will then be seen as represented in Fig. 30. The contents of the guard-cells are reduced to a minimum. Several water pores are always found but a short distance apart.

NOTES.

(1) Strasburger, *Jahrb. f. wiss. Bot.* v, p. 297; de Bary, *Vergl. Anat.*, p. 32 u. ff., 70 u. ff.; Schwendener, *Monatsber. d. Kgl. Akad. d. Wiss. in Berlin*, 1881, p. 833. In the first named authors will be found the remaining literature, at the places quoted.

(2) Westermaier, *Jahrb. f. wiss. Bot.*, Bd. xiv, p. 43.

LESSON VII.

EPIDERMIS. HAIRS. WAX AND MUCILAGE.

WE are already acquainted with the root-hairs of *Hydrocharis morsus ranæ*, and since all root-hairs are so much alike we can omit further consideration of them. We have also seen the conical papillæ or elongated epidermal cells of various petals. So also the cask-like cells forming the filamentous hairs on the stamens of *Tradescantia*, Fig. 15; also, finally, the hairs of the *Cucurbita* with its many celled base running out into a pointed filament.

The manifold aspect of plant hairs is already well known to us, but our knowledge of them should be more complete. We meet various forms of the single-celled, many branched hairs on the leaves and stems of the *Cruciferae*.

On the stem and leaves of the common wall-flower, *Cheiranthus cheiri*, Fig. 31, *A*, we have a lance-like form with the cell cavity narrow, and disappearing towards the end,

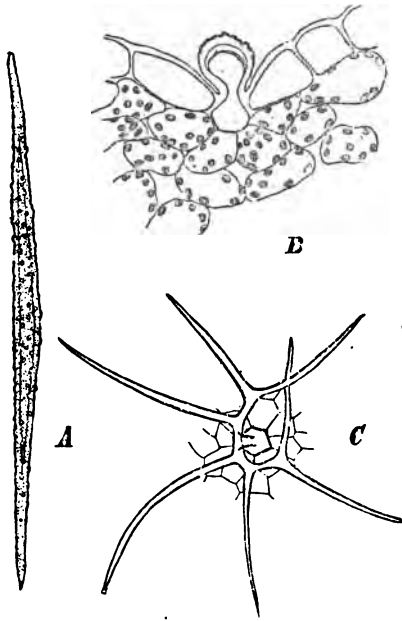


FIG. 31. *A* and *B*, hairs from under side of leaf of *Cheiranthus cheiri*; *A*, hair seen from above, $\times 90$. *B*, transection, $\times 240$. *C*, hair from under side of leaf of *Matthiola annua*, $\times 90$.

the surface thickly beset with knobs little and large. The hair lies parallel to the axis of the plant, and so a good transverse section may easily be made. But as we wish to cut it through the middle, at the point of its insertion on the leaf, a number of sections should be made in order to get just the one we want. Thus we see, Fig. 31, *B*, that the place of insertion lies somewhat deep, and that the epidermal cell which extends outwardly to make the body of the hair is slenderer than its neighbors, swollen out and rounded at the bottom and reaches deeper into the adjacent tissue, forming the "foot" of the hair. A longitudinal section shows that the foot is no wider in the other dimensions than in this, and one sees clearly that the cell cavity of the foot extends without interruption into the body of the hair. By a superficial section made through the foot, we shall find it to be circular. We also see that the cells containing chlorophyll are radially arranged about and joined to that part of the foot which is widened and extended below the epidermis.

Cheiranthus alpinus, not seldom cultivated in botanic gardens, presents the same appearance only that at one or both ends, the hair forks so that we see three or four processes all spread out parallel to the surface of the leaf.

The hairs of the stem and leaf of *Matthiola annua* are repeatedly branched in one plane, Fig. 31, *C*. These hairs are so thick upon the under side of the leaf that their branches interlock. The walls are so much thickened that the cell cavity is almost obliterated. Knobs are scarcely developed on the surface. The ball-shaped foot of the hair is considerably swollen and the cells containing chlorophyll are beautifully grouped about it in a radial manner. A superficial section turned over will show this.

The long single-celled hairs in the groove of the spur-like prolongation of the corolla of *Viola tricolor* have a

very peculiar form, Fig. 32. Make a cross section through the lower petal, close under the place where it folds up into a groove. The epidermal cells grow out into a hair for almost their entire breadth. The hairs are covered with irregular knotty swellings, and the cuticle with longitudinal projecting ridges. The cell-sap is colorless, but yellow color-bodies often occur in the wall-plasma.

The filament of the stamens of *Verbascum nigrum* is covered with single-celled violet hairs. Remove the anther and immerse the filament in a drop of water on the slide. The hair is quite long, swollen, club-shaped at the end and filled with a violet-colored cell-sap. It is covered with longish knobs arranged somewhat spirally about it.

Branched, many-celled hairs are found on the under side of the edge of the corolla. From above they have a certain resemblance to those of the *Matthiola*, but in this case every branch comes out of a common central point, and consists of an independent closed cell. It also branches not in one plane, but at all angles. Its cell walls are as thick, but there are no outside projections as in the *Matthiola*. On the edge of the leaf the hair is seen in a lateral view. The body of the hair is separated from the epidermal cell, which bears it, by a partition wall. It consists of an almost always single-celled stalk and the attached branches. The edge of the corolla also bears glandular hairs. These consist of a two- or three-celled stalk and a flattened head,

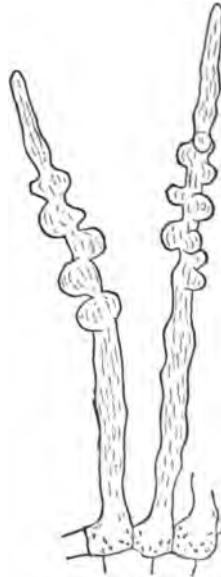


FIG. 32. Hair from the groove in the petal of *Viola tricolor*. $\times 240$.

which is occasionally covered with a highly refractive substance on its top. The latter will be studied elsewhere under more favorable conditions.

One needs only to think of the branched hairs of *Verbascum nigrum* being several times set upon each other in order to have the hairs which form the felt on the leaves of *Verbascum thapsiforme*. The hairs are five stories high, each story being separated from the preceding one by a single-celled internode which is a continuation of the principal axis of the hair. The cells are mostly filled with air.

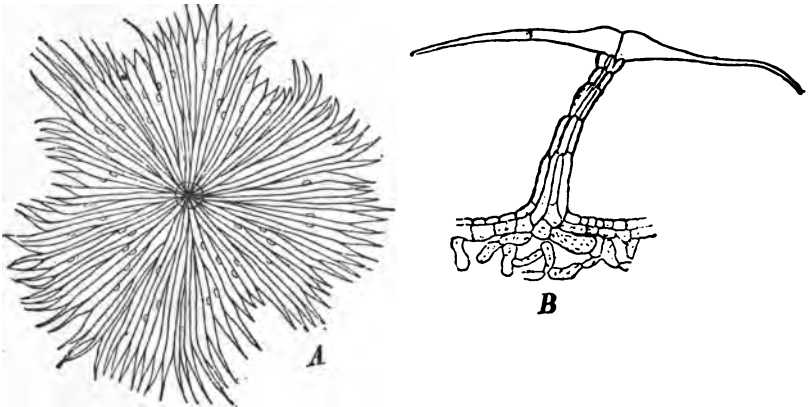


FIG. 33. Scale from the under side of the leaf of *Shepherdia canadensis*. A, surface view; B, sectional view. $\times 240$.

The best cross-section is made through the mid-rib of the leaf.

The scales of *Shepherdia canadensis* belong to the same category as the branching hairs of *Verbascum*. With the magnifying glass, we shall find white, loosely built and more closely built stars on the under side of the leaf, Fig. 33, A. On the upper side of the leaf but a few white stars are seen; the cells of these contain only air, spring from a common central point, but are laterally separated

from each other. On the upper side of the leaf the rays of the star are not confined to one plane, but radiate in all directions. The cells or rays of the brown stars are connected with each other, almost to the edge, and furnished with living contents. The nucleus is always easily seen within. A transverse section through the leaf, where it rightly hits the brown star, shows that the stalk is many celled, Fig. 33, *B*, and that not the epidermis alone, but also the next succeeding layer of cells, passes into it. The stalk bears at the top the stellate, single lamellate, many-celled expansion.

In lack of *Shepherdia canadensis*, *Eleagnus angustifolia* may, to a certain extent, be substituted. Here, only the white scales are found on the under side of the leaf, the disk consisting of cells either laterally isolated or grown together nearly to the edge.

Now, make a longitudinal cut through the stem of a rose, perhaps *Rosa semperflorens* of the garden, at a place whence a thorn projects; if possible, make it so as to divide the thorn in halves, and then make the thinnest possible section; this is not so easy to do; but do not neglect in cutting to moisten the surface of the object.

Having made a section, one can see that the epidermis of the stem continues into the thorn. The cells are more thickened and elongated. Beneath the epidermis are narrow, greatly thickened cells, and, beyond these, cells with wide cell cavities, the latter filling the whole middle portion of the spine; all these cell walls are finely perforated. The epidermis of the stem is separated from the chlorophyll tissue beneath by a layer of cells without chlorophyll, somewhat thick, elongated and joining each other with sloping sides. These cells are of like origin with those which form the inner tissue of the spine; but the tissue elements of the spine are separated from the chlorophyll tissue of the stem by a flat-celled tissue layer; this tissue

layer arises by division from the lowermost layer of the spine tissue; it follows the chlorophyll tissue of the stem but a little way and then turns towards the epidermis, so as to work off the lateral edges of the base of the spine from the chlorophyll-lacking tissue of the stem.

It is the one cork layer nearest to whose outer surfaces, by means of a separating layer, there ensues in older parts of the stem the separation of the spine; so the spine splits away from the stem, quite smoothly along the inner surface of the cork layer. Spines on the petioles lack this cork layer.

In examining the bark tissue adjoining the spine of the rose, we shall find crystals in the cells, crystals of calcium oxalate which will not dissolve with acetic acid or potash, but with hydrochloric acid without generating gas. They have the form either of a monoclinic column or of a drusen; the latter consists of a great number of crystals accumulated upon an original crystal; these crystals surprise us by their size and their stellate form.

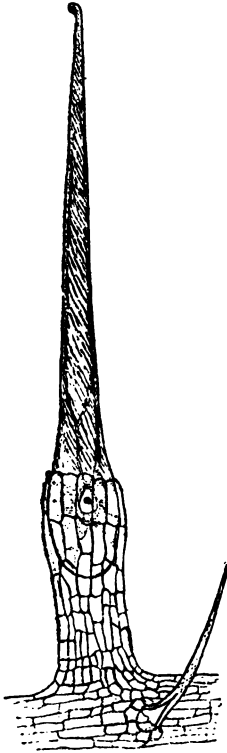


FIG. 34. Stinging hair of the nettle. *Urtica dioica*; also epidermis and small hair. $\times 90$.

In order to obtain, unharmed, the stinging hairs of the dioecious nettle, *Urtica dioica*, we must take them from the younger parts of the plant. With a razor cut a hair from the rib of a young, strong leaf and put it in water on the slide; if the hair is dead, it will be found to be filled with air, and the point is no longer intact. The uninjured hair is represented in Fig. 34; the hair is single-celled, sharply pointed and the point swollen to a small

head. At the base, the hair is broadened like a retort, and this bulb is embedded in a cup formed from the tissue of the leaf. The history of its development shows the hair to be derived from a single epidermal cell which lay at the same elevation as the neighboring cell, and the greatly swollen foot of the hair was lifted up upon a column covered with epidermal and formed of sub-epidermal tissue. In the hair itself, protoplasmic streaming may be observed. The nucleus is commonly found suspended in the bulb by plasma threads. The cuticle is covered with oblique ridges. The walls of the hair are silicated as may be shown by incinerating it on a mica plate. One often finds hairs with the points broken off. By carelessly touching the hair, its point is driven into the skin, and, as it is very brittle, it breaks off, and the strongly acid sap flows into the wound and causes a slight inflammation. A small, single-celled, bristle-like hair is seen near the other, Fig. 34, distinguished by its fine point and its thick walls; these bristles may be seen on the edges of the leaf. Put a bit of the leaf under the cover-glass in a drop of water. In all leaves the bristles will be seen from which the cell cavity has almost disappeared; their surfaces are covered with small knobs.

Glandular hairs, mentioned in connection with *Verbascum nigrum*, may be studied under most favorable conditions in *Primula sinensis*—primrose. Make a transverse section of a petiole. The body of the hair is separated from the epidermoidal foot-cells by a transverse wall outside of the epidermis and forms a cell fibre, which consists of mostly two, sometimes more, long and wide cells and one (rarely two) slenderer and shorter cells; these last cells bear the little globular head; but upon this is a cap of strongly refractive, resinous, yellowish substance. The secretion takes place between the cuticle and the cell

membrane. The cuticle is raised up, distended and finally ruptured, and the secretion is poured out over the top of the hair. An application of alcohol removes the secretion, and the raised cuticle will be seen lying in folds. The cells of the hair show a beautiful network of protoplasm with suspended nucleus in which lie large nucleoli. In the wall plasma are no chlorophyll grains.

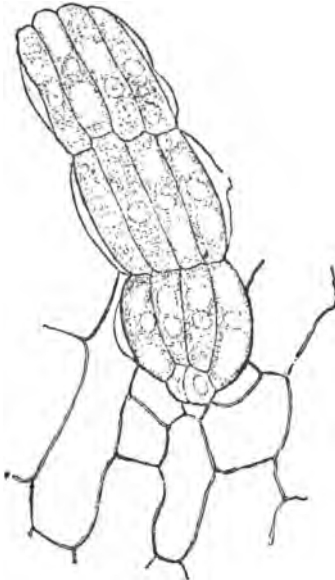


FIG. 35. Glandular tuft from the epidermal prolongation on the *Rumex patientia*. $\times 240$.

The glandular tufts on the membrane-like prolongations of the leaf sheaths of *Rumex patientia* are extremely interesting. The matter secreted by these tufts is so considerable that in moist weather the young leaves and the ends of the stems are covered with mucilage. To examine this membranous sheath direct, the inside should be turned upward. The tufts will look like small leaves, Fig. 35. These minute leaves originate with a short single-celled foot from a small epidermal cell. On the one cell are two, on these mostly four, which extending in the direction of the

long axis of the leaflet are repeated several times. On the further side-walls of the tuft are often seen bladder-like protrusions which occupy a part or the whole of the wall of a cell. The mucilage is formed here also between the cuticle and the rest of the cell membrane, and lifts the cuticle up. The bladder finally opens and lets the mucilage out. This is not colored with iodine or chloriodide

of zinc. In water it swells to a perfectly clear solution and behaves itself like a gummy body. The cells of the tuft are rich in protoplasmic contents and their nucleus is very distinct. With rose aniline violet the tuft is stained an intense violet, the mucilage mass becomes a pale red. Aqueous nigrosin solution stains the mucilage steel blue without coloring the tuft.

The structure of the glandular hairs having the function of tentacles and digestive organs of the *Drosera rotundifolia* is also very interesting. They arise from the edges and the whole upper surface of the leaf in the form of slender filaments attenuated upwards and expanded into an egg-shaped termination, Fig. 36. They are constructed of delicate elongated cells, the larger hairs penetrated throughout with one or more spirally thickened tubes, the spiral vessels. The radial extension of the epidermis to form the head of the hair, the superficial arrangement of the epidermal elements and their increase till they consist of three layers, may be best seen in an optical section of the object, Fig. 36. The number of the spirally thickened cells are greater in the head of the hair. All cells which lie inside the envelope, formed by the division of the epidermal cells, are spirally thickened. By examination of the place of insertion, we shall see that not only the epidermis but also the inner tissue of the leaf is continued into the hair. These digestive glands secrete a slimy fluid which clings to the top of the little head like a drop of dew. It is not produced from beneath the cuticle but on



FIG. 36. Digestive gland of *Drosera rotundifolia*. $\times 60$.

the free outer surface. Small insects get caught and stuck fast in this slimy drop and then by the bending down of the hairs they are carried to the middle of the leaf. Now other hairs also bend over and touch the insect body with their tops. Immediately the chemical qualities of the secretion change, a free acid and a pepsin-like ferment being produced which slowly digests the insect or any other albuminous body. The digested substance is then absorbed into the plant.

A transection through the winter bud of the horse-chestnut, *Æsculus hippocastanum*, shows us button-shaped glandular tufts upon the covering scales, Fig. 37. The middle

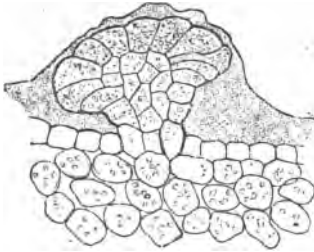


FIG. 37. Glandular tuft from an enveloping scale of the winter bud of *Æsculus hippocastanum* surrounded by its secretion. $\times 240$.

scale has these tufts on both sides, but the outside scales have these more on the inner surface, and the inside scales more on the outer. The structure of the tuft is seen from the figure; a series of cells in the middle divide above and from these the secreting cells radiate.

The illustration shows a longitudinal section of the tuft. The cuticle will burst and the secretion will be poured out between the scales coating them over and gluing them together. This secretion is a mixture of gum and resin. The minute drops of gum swell in water, while rose aniline violet colors the resin masses a beautiful blue. The contents of the tufts become red.

We have already remarked the fine-grained waxy coating upon the epidermis of *Iris florentina*. We will now investigate this point in some other plants.

A suitable plant is found in *Echeveria globosa*. The waxy coating gives the plant a hoary or glaucous appear-

ance, and may be easily rubbed off from the leaf. An inspection of the upper surface of the epidermis shows us a net-like crust of blended grains.

A wax coating, in the form of short rods massed together, may be easily observed on the epidermis of *Eucalyptus globulus*.

Saccharum officinarum is also a beautiful object. Here the wax coating appears in the form of long rods often curled at the ends. Prepare a superficial section from a node of the stem noticeable for its glaucous appearance. To remove the air from between the rods dip the section in

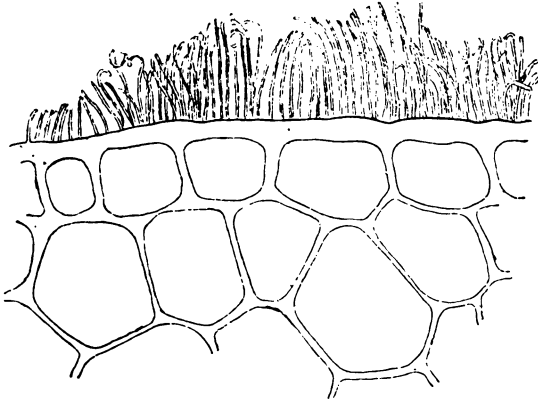


FIG. 38. Transection through a node of the stem of *Saccharum officinarum* showing a rod-shaped wax coating. $\times 540$.

cold alcohol for a short time. It is difficult to make a transection to which the little rods still adhere. Such an one is shown in Fig. 38. The rods stand closely pressed together, and show the before mentioned curling. Brought near a flame the rods melt as they also do in hot alcohol.

NOTE.

(1) See in de Bary's *Vergl. Anat. die* §§ 10, 13, 16, u. ff. and there also for the literature.

LESSON VIII.

CLOSED COLLATERAL VASCULAR BUNDLES.

THE common cornstalk, *Zea Mays*, furnishes an excellent object for studying the structure of the closed collateral fibrovascular bundles of the monocotyledons (1). An alcohol specimen should be used for studying the cell contents. Make a transection through an internode and put it on a slide in a drop of chloriodide of zinc and lay the slide on a piece of white paper. We may now with the naked eye see the bundles, in the form of oval-shaped dots, and their arrangement characteristic of the monocotyledons. There is no specialization of pith and rind by the distribution of the bundles. With a low power, select for examination a bundle lying not too near the surface of the stem, noting which way the latter lies so as to distinguish the inner and outer edge of the bundle. The bundle is represented in Fig. 39. The sheath, *vg*, consists of thickened lignified parenchyma cells, surrounds the bundle, and is stained red-brown. The intercellular passage, *l*, is surrounded with narrow thin-walled cells colored yellow by the zinc reagent. The ring, *a*, belongs to a ring vessel, for the most part ruptured by stretching. The intercellular passage may be produced by either of two ways, by the rupturing of the cells or by their separation from each other. The one we may call the "lysignian," the other the "schizoginian," method. The ring vessels and some other which sometimes project into this passage are the first elements formed in the fibrovascular bundles when the plant is rapidly growing in length. Upon the

FIBRO-VASCULAR BUNDLES.

outer edge of the bundle are one or more vessels
 this case, *sp*. This vessel has spiral walls as v
 able to see by a longitudinal section. Further
 right and left are two wide, open spaces, *m*, *m'*, v
 pitted walls. A ring or part of one is often seen project-

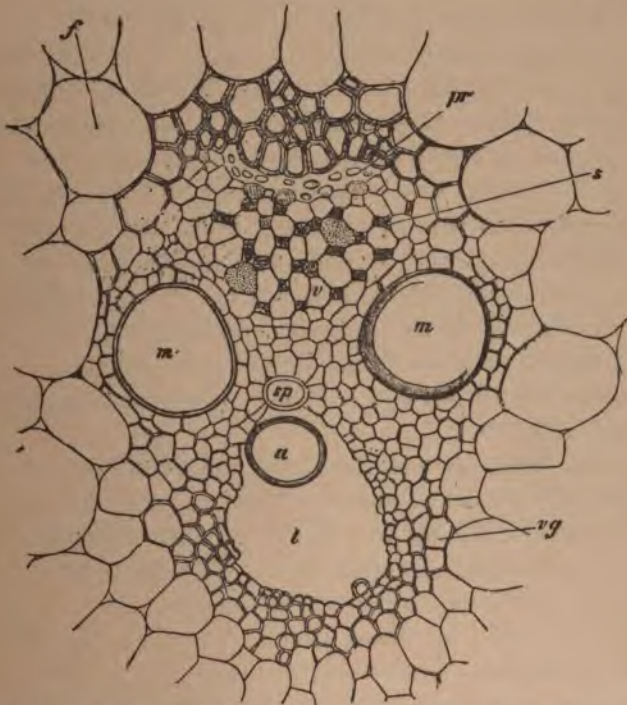


FIG. 39. Section of a fibro-vascular bundle from the inner part of a corn stalk, *Zea Mays*. *a*, joint of a ring vessel; *sp*, spiral vessel; *m* and *m'*, pitted vessels; *v*, sieve tubes; *s*, conducting-cells; *pr*, compressed protophloëm elements; *l*, intercellular passage; *vg*, sheath. $\times 180$.

ing into the interior of these vessels, *m'*. It is all that remains of a perforated division wall once existing intact between two cells. The cells lying between these large vessels are reticulated. The walls of the vessels, espec-

ially of these, are stained a yellow-brown. The cells between the vessels are stained a darker yellow than those about the intercellular passage.

The above described portion of the bundle is called the woody or xylem vascular part; this does not imply that these cell walls are very much thickened. The woody part or vessels are never lacking in the bundle, hence the term xylem is morphologically correct. In the example just studied, we have touched upon the woody portions, viz., the xylem, the protoxylem, the wood parenchyma and the vessels of the fibrovascular bundles.

For the other or secondary element of the bundle, we select the term bast or phloëm. Since the sieve-tubes never fail in the bast, it is morphologically more reasonable to call the bast the sieve part (2). Vessels and sieve tubes constitute the fibrovascular bundles, and, being laterally united, they are called collateral bundles. By including the sheath also, we may call the whole a fibrovascular cord (3).

A solution of the chloriodide of zinc colors the bast in this bundle a distinct violet. Wide and narrow unligified cells regularly alternate: the former are sieve tubes, *v*; the latter conducting-cells, *s*. We shall see the fine, sieve-like punctures when the section is made at or near a division wall (see illustration). On the outer border of this group are a number of thick-walled cells, *pr*; they were the first produced, but their activity terminated in the sieve-tubes and conducting-cells; they are the beginnings of the bast, the protophloëm elements, and are stained brownish. The cells of the sheath border upon these, the inner ones having wide cell cavities, but the outer sclerenchyma cells of the sheath gradually pass over through intermediate forms into the large-celled parenchymatous, fundamental tissue; the cells of the latter

are stained yellow with an occasional violet tinge. Towards the outer surface of the stem the bundles are more closely packed, the intercellular passages disappear, the elements are reduced one by one, and the sheath is greatly enlarged. Lateral unions between large and small bundles are frequent in this part of the stem, the conjunctions taking place on the sides at the points where the large vessels are.

Enclosing the epidermis of the stem is a stout ring of tissue, like that of the sheath, and gives the same reaction with chloriodide of zinc. This extreme outer layer is called the hypoderma and is unbroken except by the stomata. The hypoderma and the bundle sheath protect the thin-walled tissue and give stability to the stem; comprehensively, they are the "stereïds" and form the mechanical-tissue system, the "stereöms" (4). Mechanical principles require that for stiffness the stereöms be placed as near as possible to the periphery of the stem. The peripheral bundles, including the sheaths, constitute a system of compound pillars. The sheath is the column, the bundle is its feeling. The hypodermal cylinder is strengthened by the sclerenchyma tissue of the sheaths, even if, as in this case, it is not much developed; this cylinder consists of a number of interblended columns placed in a circle.

Coralline soda (5) rapidly stains the lignified walls in both the bundles and fundamental tissue a bright, coralline red, and the unligified a rose color. This brings into great prominence the sheath cells, the walls of the vessels and the epidermal ring.

Now, make a number of radial sections, so as to be sure to have one at least through the middle of a bundle. It should show the bast and the ring vessels; stain with chloriodide of zinc; the colors will correspond to those of the

transection. But a coralline-stained section will be better for study, Fig. 40. Begin at the inner edge of the bundle. We first meet the nearly cubical cells of the fundamental tissue, and then the sheath cells of the bundle, *vg*, deeply-stained, elongated, somewhat pointed, and dotted with slit-like pits diagonally arranged. Within

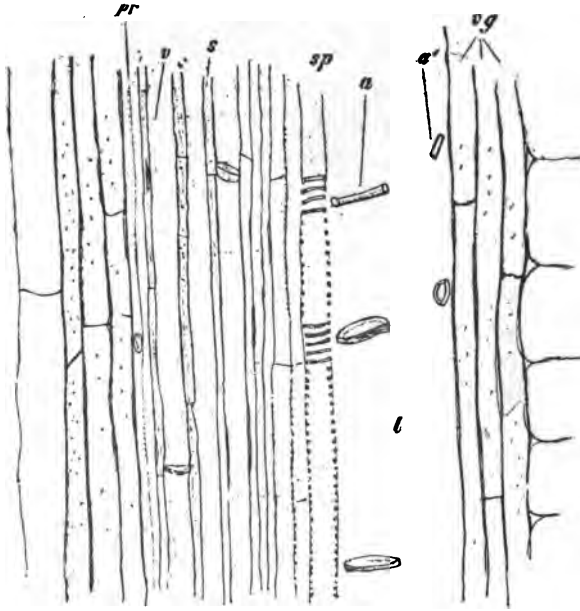


FIG. 40. Longitudinal section of corn stalk, *Zea Mays*. *a* and *a'*, joints of a ring vessel; *sp*, spiral vessel; *v*, sieve tubes; *s*, conducting-cells; *pr*, protophloëm; *l*, air passage; *vg*, sheath. $\times 180$.

these elongated sclerenchyma cells is a much reduced wall layer of protoplasm and a nucleus. Next to the sheath is the intercellular passage which extends through the whole length of the bundle. It is surrounded with primary, wood-parenchyma cells, thin-walled, shorter than those of the sheath, have more cell contents and are separated by transverse walls. Isolated, small rings, *a*, be-

longing to a ring-vessel which has been ruptured during the elongation of the internode are seen attached to the outer side of the passage; these and other small rings, sometimes seen, *a'*, constitute the remainder of the protoxylem elements. Next to the rings are one or more spiral vessels, *sp*; only one, and that a narrow one, in this case. Next, are somewhat, thicker-walled, relatively short, wood parenchyma cells, with pitted and mostly reticulated walls; beyond these are the bast cells, recognized by their thick, rose-colored division walls, the sieve-plates of the sieve-tubes, *v*, the punctures of which may be seen with a high magnifying power. The conducting cells, *s*, are placed side by side with the sieve-tubes, are slenderer, shorter, and have a nucleus which the sieve-tubes have not. The sheath cells bound the vascular bundle and are so acutely pointed that we may speak of them as vascular fibres. Starch grains are not found in the cells of the vascular bundles nor in those of the fundamental tissue. All these cells except the vessels and the sieve-tubes have a nucleus.

A longitudinal section through the middle of the bundle will not show the larger vessels, except in some cases by deep focussing, and then indistinctly. To see these, make a section through the side of the bundle. They are obliquely pitted. The pits are enlarged at the bottom but bordered only on one side, as the corresponding pits of the adjacent wood-parenchyma cells have no borders. The diaphragm, which consists of a double ring projecting into the interior of a vessel, is produced by the thickening of the outer edge of the transverse wall of the cell, and the subsequent absorption of the thin middle partition.

We should now make a permanent preparation of both the transverse and longitudinal sections. Stain with saffranin or iodine green. For double staining, immerse for

a considerable time in iodine green and then in Grenacher's carmine (6). For instantaneous double staining use picro-

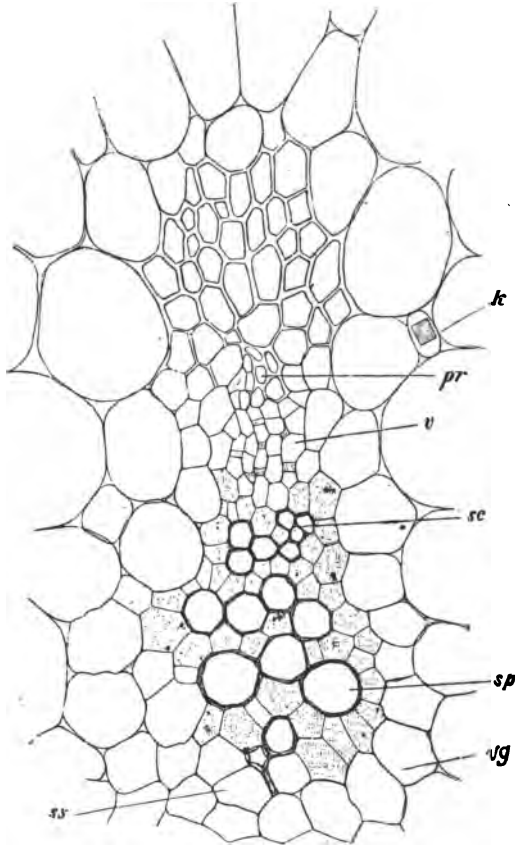


FIG. 41. Transection of fibro-vascular bundle from leaf of *Iris florentina*. Elements with dark outlines are vessels and the shaded ones are those rich in cell contents. *ss*, compressed spiral vessels; *sp*, wide spiral vessels; *sc*, scaliform vessels; *v*, sieve tubes and between them the narrow conducting-cells; *pr*, compressed protophloëm elements; *vg*, sheath with wavy radial cell walls; *k*, section of a crystal. $\times 240$.

nigrosin, or picro-aniline blue. The iodine green and the picric acid will color the lignified cell walls; the carmine,

nigrosin and aniline blue stain the unlignified walls and the cell-contents. Mount in glycerine or glycerine jelly. If in the former, clean away all the superfluous glycerine and cement the cover-glass down with a solution of Canada balsam in turpentine or chloroform, of the consistency of syrup. Use a glass rod as thick as a match for this purpose. Gold size or varnish will not answer. Glycerine-jelly mounts do not need to be sealed.*

If a cornstalk is not available for this examination use the stem of *Avena sativa*, common oat, or any other *Graminaceæ*.

We will next make a longitudinal and transverse section of an alcohol specimen of the full-grown leaf of *Iris flor-entina*. The alcohol specimen cuts better, contains no air and has the cell-contents already fixed. Let it lie for some time before cutting in a mixture of glycerine and alcohol. Stain the section by immersion for several hours in borax-carmine, and a short time in iodine green. The cell-contents will take the carmine. The lignified walls and vessels will be stained green, also commonly that part of the sheath which lies next to the bast. The protophloëm elements will be blue. Such a section is represented in Fig. 41. The cells with red cell-contents are shaded in the illustration. The green walls of the vessels are dark, and the blue protophloëm cells are light. The thickened cells of the fundamental tissue are unlignified and hence uncolored. For instantaneous staining use only iodine green, and if only the lignified walls are to be stained the exact time for the effect must be carefully determined. The cell-contents in that case will not be stained.

Our examination will proceed from the wood towards the bast elements from the inner and upper to the outer and

*But they may be more conveniently handled, and are less liable to injury if they are securely cemented and nicely finished.—A. B. H.

lower side of the leaf. The number of vessels is pretty large in the wood part and they increase in size towards the bast. They either touch each other, or are separated by thin walled, relatively narrow, primary wood-parenchyma cells full of cell-contents. These cells also surround the vessels and separate them from the fundamental tissue. The compressed cells, *ss*, are protoxylem elements. In the bast the wide cells are the sieve-tubes, and the small ones, rich in contents, are the conducting-cells. Beyond, at *pr*, lie the protophloëm elements, stained blue. This part of the bast is surrounded with the much thickened sclerenchyma of the sheath. It is wanting about the rest of the vascular bundle. The cells of the fundamental tissue are smaller about the vascular bundle, and have no intervening air spaces. There are several intermediate forms between these cells and the larger ones of the fundamental tissue with air-filled intercellular spaces. At *k* is a small cell containing a highly refractive crystal.

By the use of coralline, we shall get a good and rapid staining, the lignified sclerenchyma becoming a fiery red, the as yet unligified a bright rose color, the vessels a brown red and the other elements a pale yellow red.

For comparison we will prepare a section from a fresh leaf. We see that the outer large fundamental cells contain chlorophyll; those next the bundle do not. The vessels contain air which somewhat impairs their microscopic image. The radial walls of the first layer of cells next the wood part of the bundle seem to have dark broad pits. By looking at the other section again we see that these walls are arched towards one side, *vg*, Fig. 41. By focusing up and down we shall see that this arched part of the wall forms a wave-like band bent back and forth. As we shall meet a similar structure elsewhere we will spend no more time on it now.

A longitudinal section cut through the middle of a

bundle shows on the inner edge of the bundle a much elongated, partly compressed, spiral vessel, Fig. 41, *ss*; this is the protoxylum element. Beyond are closely wound spiral vessels, and, further along, narrow, scale-formed vessels. In the bast the sieve-plates show plainly only in the coralline preparation. Further towards the outside are the sclerenchyma fibres distinguished by their considerable length, thickness and pointed ends.

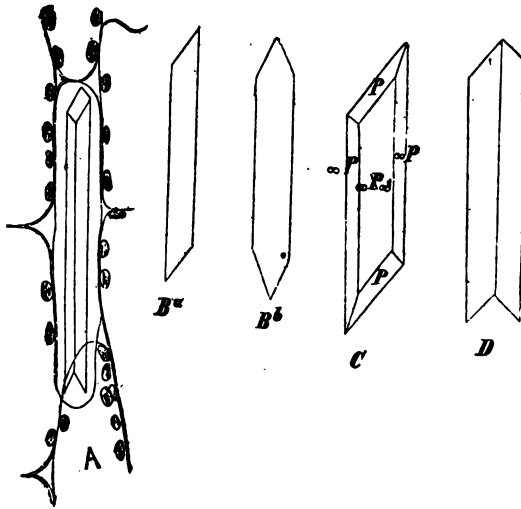


FIG. 42. *A*, crystal of calcium oxalate in a cell of the leaf of *Iris florentina*. $\times 240$. *B*-*D*, figures which explain the form of the crystal; *B*¹ and *B*², and *D*, the optical section; *C*, projection on a symmetrical plane.

In the longitudinal section, the crystals are seen in profile, lying parallel to the longer axis of the leaf, Fig. 42, *A-D*; they are found in elongated cells of the fundamental tissue, the crystals being nearly as long as the cells; these cells have no chlorophyll like most of the neighboring cells. The crystals are calcium oxalate and dissolve without generating gas in muriatic acid; they have a long prismatic form, are mostly twins, *D*, and be-

long to the monoclinic system. The cell-contents of these cells are not stained with the coralline.

The vascular bundles of the monocotyledons are mostly built on the type of these two cases. Closed, vascular bundles

are not well adapted to the lateral growth of monocotyledons. This growth, which is limited to plants of the families of *Dracæneæ*, *Alionææ* and *Dioscuraceæ*, takes place by means of a cambium zone lying outside of the vascular bundles.

We will select *Dracæna rubra*, cultivated in every market garden, and make a transection of the same. Inside the brown cork layer, we see with the naked eye a green, soft rind about 1 mm. thick, against which is contrasted the yellowish hard tissue of the stem. On the border of this is the cambium ring. The circular centre of the stem has a lighter color.

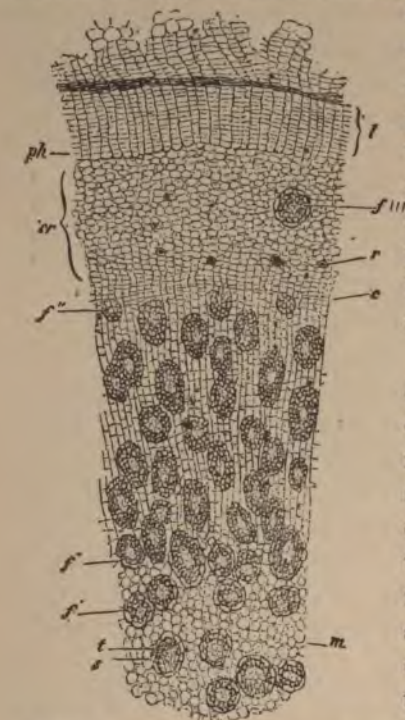


FIG. 43. Transection of stem of *Dracæna rubra*. *f*, vascular bundle; *f'*, primary, *f''*, secondary bundles; *m*, unligified fundamental tissue; *s*, lignified fundamental tissue, surrounding the bundle; *t*, tracheids; *c*, cambium ring; *cr*, rind; *l*, cork; *ph*, cork cambium; *r*, bundles of raphides. $\times 30$.

Now put the section under the microscope, with a low magnification, Fig. 43. The central fundamental tissue of roundish cells, *m*, has in it isolated round or oval vascular bundles, *f'*. Beyond a certain point, *f''*, they are more numerous, compressed

and crowded together so as to touch, or are separated by only a single layer of fundamental tissue. In the latter, the cells are much thickened, deeply pitted, radially elongated, and arranged in a radial series. Beyond this we come to the boundary between the yellow tissue and the green rind, *c*, a zone of flattened, thin-walled cells arranged in a strictly radial order. This is the cambium ring which provides for the lateral or radial growth of the stem. It belongs apparently to the fundamental tissue. In the middle of the zone is the initial layer of cells, one cell thick, whose successive cell divisions produce the new elements. These divisions, occurring tangentially, produce the radial arrangement of cells. An occasional radial division occurs also, and so starts a new radial series of cells. Vascular bundles occur in the young tissue in all stages of development. The youngest consist of a group of thin-walled cells. The older ones are complete on their inner edge already, but on the outer are still in the process of formation.

It is supposed that from the point *f''*, the tissue is secondary, produced by the activity of the cambium ring. The rind, *cr*, consists of roundish cells. Crystal needles in groups or in bundles are found in the cells of the inner border of the rind, principally. These so-called "raphides" are calcium oxalate. The other cells of the rind contain chlorophyll grains. The bundles, one of whose round sections are seen at *f'''*, are those provided for the leaves. The stout layer of thin-walled, colorless, radially-arranged cells, *l*, which pass outwardly into a brown, less regular tissue is the cork layer; on the inside, young, colorless, and on the outside, old, irregularly elongated and browned cork-tissue.

Stain a transverse section with coralline. The bundles come out sharply. The lignified, secondary, fundamental

tissue is stained another shade of red, the unligified, a pale rose red. The raphide cells seem filled with a red liquid. So we know that the raphides are embedded in a homogeneous mucilage which absorbs coralline. This reagent stains vegetable mucilage; neither cold nor hot alcohol will discolor this mucilage thus stained. But mucilage derived from cellulose is bleached in both hot and cold alcohol (7). In this we have our test of starch and cellulose mucilage. Gum is not stained by coralline. A mixture of gum and mucilage is or is not, according to conditions. An aqueous solution of nigrosin will not stain the mucilage of this plant, even with long treatment, but it will that of *Rumex*.

This survey of the transection shows us the process of the radial growth of the plant. We will omit the study of details and of the longitudinal section.

NOTES.

(1) In regard to the vascular bundles generally, see de Bary, *Vergl. Anatomie* 1877, especially Chapter VIII, where the whole of the older literature may be found. Numerous critical investigations of the morphology of the vascular bundles, which have recently appeared, have not had a coherent treatment. G. Haberlandt, on the contrary, in the *Encyklopädie der Naturwissenschaften, Handbuch der Botanik, Bd. II, p. 593*, has accomplished this in part by attempting a physiological interpretation of morphological facts, in his physiologico-anatomical works.

(2) The designation vascular part and sieve part was introduced by de Bary, *Vergl. Anat.*, p. 330.

(3) See Haberlandt, in the history of the development of the mechanical tissue-systems of plants.

(4) Schwendener. The mechanical principles in the anatomical structure of the monocotyledons.

(5) This staining fluid was introduced by Szyszyłowicz. See *Bot. Centralbl. Bd. XII, p. 138*.

(6) See Tangl, *Jahrb. f. wiss. Bot.*, Bd. XII, p. 170.

(7) See Szyszyłowicz at the same place.

LESSON IX.

OPEN COLLATERAL VASCULAR BUNDLES.

FOR our study of the open collateral bundles of the dicotyledons we will take a runner of *Ranunculus repens*. Stain the section with coralline. We find the bundles isolated from each other and arranged in a simple circle in the stem. The fundamental tissue consists of rounded cells which diminish towards the surface of the stem, contain chlorophyll and have large intercellular spaces between them.

At the surface is the epidermis, but within, the stem is hollow, caused by the separating and rupturing of the cells. The vascular bundles have the same appearance as those of the monocotyledons, the same parts appearing in the same order. The woody part consists of vessels and thin-walled parenchyma cells; the ring and spiral vessels on the innerside of the bundle next the vessels take up but little coloring matter, Fig. 44, *s*. The other subangular vessels are colored brown-red. In these walls are bordered pits, *m*. Between these vessels lie the soft-walled primary wood parenchyma. In the bast is again the alternation of large sieve-tubes and small conducting cells. But the bast is separated from the woody part by a many-layered stratum of radially-arranged cells, *c*. This arrangement betrays their cambium origin. A cambium layer separates the woody from the bast part, and distinguishes this from the monocotyledons. The activity of this cambium layer is indeed limited outwardly, but there is enough of it to give the bundle a place among the "open bundles," that is, those capable of a lateral growth.

It has formed a stratum several layers thick of thin-walled cells and then ceased to grow. The bast is protected on the outside by a cord of sclerenchyma cells, colored a beautiful coralline red. So also the inner edge of the bundle is inclosed by another but thinner-walled layer of such sheath cells. The sheath cells do not meet at the sides of the bundle.

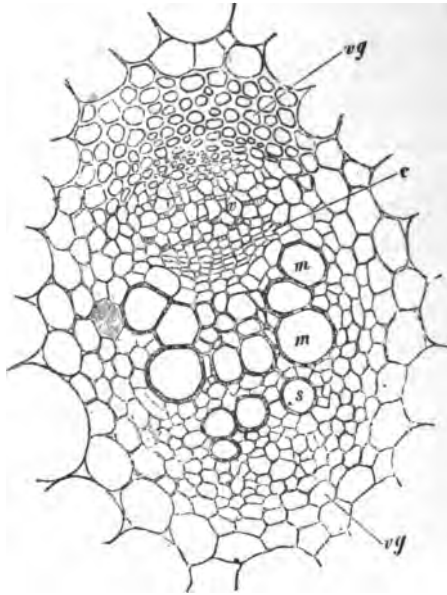


FIG. 44. Transection of the vascular bundle of a runner of *Ranunculus repens*. *s*, spiral vessels; *m*, bordered pitted vessels; *c*, cambium; *v*, sieve-tubes; *vg*, sheath. $\times 180$.

In the longitudinal section, ring, spiral and pitted vessels, and between them elongated wood parenchyma cells, are easily made out. Then follow thin-walled cambium cells, sieve-tubes, conducting cells, and finally sheath elements with but slightly inclined, porous, transverse division walls.

The vascular bundles of celandine, *Chelidonium majus*, are so like those of the *Ranunculus repens* that a cross-section can be easily understood from that. We prefer alcohol material. The wood part shows large vessels pressed close together which in the older stems have yellowish walls. The bast is strongly developed; between the two lies the stratum of thin-walled, radially-arranged cells produced by the activity of the cambium. The sheath appears only in a bundle of thick-walled sclerenchyma cells on the outer border of the bast, in old stems colored yellow. Running around, just within the epidermis, and separated from it by a cell-layer two cells thick, is a ring of sclerenchyma cells, like those which protect and support the bundles, making a common sheath to the whole inner tissue of the stem. In and on the bundles we meet an element not heretofore seen, the milk-tubes. They are filled with a dark brown substance, which is the orange-red milk-sap of the plant into which the alcohol has run.

They are found in the bast of the vascular bundles, also on the inner border of the wood part; especially numerous on the sides of the bundles and the outer edge of the sclerenchyma tissue, and scattered singly through the fundamental tissue between the bundles. They are all thin-walled, even those intercalated in the outer edge of the sclerenchyma tissue. It is impossible not to see them. In the longitudinal sections they are easily recognized also by their yellow-brown contents. They run parallel to the axis of the stem, are furnished with transverse walls which are perforated with one or more pores, and yet these walls are quite lacking sometimes in places where we expect to find them. Often some of the vessels in the bundles are found filled with coagulated milk-sap.

Stain a transverse section with coralline, then apply a drop of potash lye to the edge of the cover-glass. The

vessels will appear fuchsin-red, the sclerenchyma cells rose-red, while the milk-tubes and their dark brown contents will come out very distinctly. Put a very thin longitudinal section in 45 per cent acetic acid carmine, and nuclei may be detected in the milk-tubes, but not very easily. Lateral unions of the milk-tubes have not been seen in this plant.

Aristolochia siphon, or Dutchman's pipe, affords an uncommonly good object in which to study the lateral growth of the dicotyledons. Make a section of a branch 3 to 4 mm. thick. With a lens observe the inner loose pith; about this a circle of isolated vascular bundles; about this a continuous white ring; then green rind-tissue; finally, a yellowish-green peripheral envelope. With a low power under the microscope we see that the pith is composed of large round cells in part filled with air. In the vascular bundles the wood part is darker and much broken by the wide cavities of the vessels. The cambium zone follows, composed of narrow, radially-arranged light cells, and here-upon the bast cells, not so light nor so regularly arranged but much larger. Each bundle is bordered about, especially on its outer part, by parenchymatous tissue containing some chlorophyll grains, and eventually also reserve substances. The white ring beyond is formed of much thickened sclerenchyma cells which project inward somewhat in wedge-shaped masses between the vascular bundles. Abutting upon this ring is the tissue which contains chlorophyll and air-filled intercellular spaces. Beyond this comes narrow-celled tissue containing chlorophyll, the cell-walls white and thickened at the corners, on account of which they are called "collenchyma" cells. Finally, the epidermis.

Now, make a very thin section of a bundle from alcohol material, that has previously lain in a mixture of equal

parts alcohol and glycerine. Stain, by immersing for some time in coralline. Fig. 45 gives a representation of a section of a bundle made from a growing, this year's branch, about the beginning of June. The vascular bundle begins on the inner edge with thin-walled, primary wood paren-

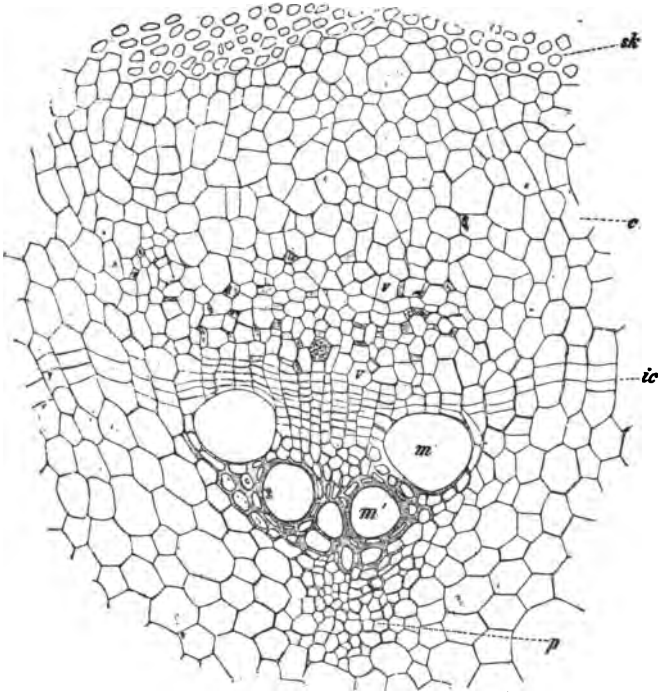


FIG. 45. Transection through a young twig of this year's growth of *Aristolochia siphon*, showing a vascular bundle after the beginning of the active growth of the cambium. *p*, parenchymatous elements on the inner edge of the wood part; *m*' and *m*'', vessels with bordered pits; *ic*, interfascicular cambium extending into the fascicular cambium, that is, into the cambium within the vascular bundle; *r*, sieve tubes; *c*, rind-parenchyma; *sk*, inner part of the ring of sclerenchyma fibres. $\times 130$.

chyma, *p*, in which narrow vessels, which gradually become wider (protoxylem elements), are inclosed, the cells themselves also becoming thicker-walled. The same also may be said of the vessels, while the intervening spaces are

taken up by tracheïds, with thickened and border-pitted walls. The thick-walled wood parenchyma, vessels, and tracheïds take an intense red, while the thin-walled are colored a faint rose. The two largest vessels are seen in the process of development. Between these lies thin-walled, serially-arranged, secondary tissue, of cambium origin. On the outer border of the two large vessels is the cambium zone, in which an especially flat, not very sharply defined cell-layer represents the initial stratum. Following this, are like cells of the bast, the radial arrangement of which betrays their secondary cambium origin. In the middle of the bast are the sieve-tubes from which the accompanying conducting cells are distinguished by the contents existing in a majority of them. The outer part of the bast, the protophloëm, is taken up by narrower sieve-tubes, which are, therefore, not so sharply contrasted with the conducting cells. The bast is separated from the sclerenchyma ring, *sk*, by large-celled rind-parenchyma. The sclerenchyma is as intensely colored as the wood part of the vascular bundle. The protophloëm elements are compressed by the multiplication and growth of the new cells from the cambium. Such a section shows very perfectly the formation of the interfascicular cambium.

With the beginning of the activity of the cambium in the vascular bundle, the cells of the fundamental tissue adjoining the same at the sides also increase by self-division through the introduction of dividing walls, *ic*. So the elements of the fundamental tissue are formed into a cambium stripe which unites the cambium layer in the circularly placed vascular bundle into a continuous cambium ring. As the figure shows, it is very easy to follow the formation of the interfascicular cambium in this plant, and to recognize for a long distance the original outline of the divided fundamental-tissue cells. The sheath is altogether lacking in this plant about the single vascular bundles.

The sclerenchyma ring forms a common sheath about the whole inner tissue of the stem. A radial longitudinal section cut through the exact middle of a vascular bundle and stained with coralline shows, on the innermost side, elongated primary wood parenchyma with transverse division walls, between which are very narrow, somewhat compressed ring vessels; then somewhat wider ring vessels, which in part pass into spiral vessels; then closely spiralled wider vessels which pass into reticulated vessels; finally, the enlarged border-pitted vessels. Between these vessels are much elongated, border-pitted, empty tracheids; single fibre cells, like the tracheids in form, but having unbordered pits and filled with starch; thick-walled wood parenchyma, shorter, with transverse walls, likewise unbordered pits and starch. The incomplete, large vessels are still wide, cylindrical, thin-walled cells, separated by transverse division walls, with rich protoplasmic wall-lining, and a nucleus. In the fully completed vessels, nothing of their cell-contents is to be found, and of the division wall, in the pitted vessels, there remains only the ring-like projecting diaphragm. The flat cells of the cambium zone are rich in protoplasmic contents, nucleus and delicate transverse division walls. The sieve-plates are quite extraordinarily beautiful. When they are somewhat inclined, they present to the observer their whole rosy surface with dark, sparkling points. In those sieve-plates which are much inclined, the plate is divided by clear belts without pores, into several rose-colored, dotted and superimposed sections. The side walls of the sieve-tubes are also covered with small, transversely extended, finely dotted, rose colored sieve-pits. In the periphery of the bast occurs, in the most astonishing manner, the formation of the callos-plates, those bright, rose-colored, strongly refractive masses, which are rounded upon the free side, and which

cover both sides of the sieve-plate in like mass, or are preponderatingly on one side only. The sieve-pits on the lateral walls also have minute callus-plates. We also find here with the sieve-tubes, the narrower conducting-cells filled with their contents. The bast is separated from the sclerenchyma ring by the broader—and, as this section shows, also relatively shorter—parenchyma cells. The sclerenchyma fibres of the ring are very long, pointed at their ends, comb-like with their ends interlocked and provided with pores. And, finally, we notice that the collenchyma cells bordering on the epidermis, are several times longer than broad and are joined with transverse walls.

Now cut a section from an older branch, say one 10 mm. in diameter and examine it with the lens. The pith and the medullary rays are white, the wood-bodies yellowish.

The thickest medullary rays, some ten or twelve in number, open into the pith, and are those primary rays which in the beginning separated the vascular bundles. The oldest wood part of the bundles border on the pith. Since the wide vessels are lacking in them, they seem to be a thicker, darker ring penetrated by the primary medullary rays. To these succeed the concentric yearly rings. The width of the vessels increases in the first year's growth till it reaches a definite greatest diameter. The boundary of the yearly ring is clearly marked by the larger vessels, since those of widest cavity are produced in the beginning of the development in the spring. The outer part of the yearly ring contains no vessels distinguishable with the lens. As the secondary wood-bodies increase in circumference, new medullary rays are intercalated which we designate as secondary rays of the second, third, fourth, etc., orders. The intercalation of secondary rays follows with the greatest regularity. The farther we go from the centre, the more numerous are the medullary rays and the

shorter are the newly added ones. On the outer border of the wood body we see the cambium ring as a dark circle, the medullary rays within which are indicated by delicate lines. Before the secondary wood parts, we see the clear brown-colored secondary bast lying, formed from successive growths. The medullary rays extend beyond the cambium in consequence of its supplementary lateral growth caused by the increase of the thickness of the stem. The bast is not capable of this supplementary lateral growth and appears thence from the outside to be narrowed and rounded. The original continuous sclerenchyma ring is dispersed in single olive-green colored pieces of unlike size; likewise, also the original continuous darker olive-green collenchyma layer. The periderm now undertakes the protection of the interior, and as a brown, distinctly laminated sheath covers the surface of the stem. The whole of that part subsequently produced by the cambium, which includes the secondary bast and the extended medullary rays, becomes secondary rind, which confronts the primary rind previously existing before the beginning of the lateral growth of the stem. No sharp boundary exists between the primary and secondary rinds.

Now apply a higher power to the investigation of a thin cross-section of this stem. The pith tissue is unchanged from its young state, only that it has numerous crystal masses of calcium oxalate. The primary wood parts project into the pith tissue, forming the so-called "medullary crown" or "medullary sheath." The hand-lens will not show this, as the inner parts are composed of thin-walled compressed cells. First on entering the solid part we find the wood-bodies clearly marked off from the large pitted vessels. The vascular bundles increase in breadth correspondingly to the narrowing of the medullary rays. The vessels formed in the spring, up to the third or fourth annual ring, show an

increase in volume. From the spring toward the fall, the width of the vessels rapidly decreases in each annual ring. Shortly before the close of the year's growth, only very narrow vessels are produced. The great mass of the wood consists of tracheïds, narrow, thickened, empty, border-pitted cells. They contain air or water. If starch grains are ever seen in them, the knife has carried them there from neighboring cells. Distributed about the circumference of the vessels mainly, but also among the tracheïds, are thinner-walled cells with protoplasmic contents; also, commonly, starch and flat pits. These are wood-parenchyma and fibre cells. The vessels are provided with bordered pits, only when they touch each other or the tracheïds. When a vascular-pit or a tracheïd-pit meets the pit of a wood-parenchyma or fibre cell, it is one-sided, that is, bordered only on the side of the vessel or tracheïd, or, so to say, narrowed in its opening only on this side.

The closing membrane of such one-sided pits is without central thickening (torus) and, unlike such thickened membrane, is colored blue with chloriodide of zinc (1).

The cells of the medullary rays are radially extended, relatively thin-walled, and have numerous pores. On the outer border of the wood substance, we easily recognize the cambium formed from flat, thin-walled, radially-arranged cells, and beyond that the thin-walled bast. Besides sieve-tubes and conducting-cells, we find in this also starch-bearing bast parenchyma.

The secondary bast produced by the cambium has consequently gained the latter additional elements. With an extremely delicate section one can follow in the bast the alternation of uncompressed with fully compressed cell layers. Similarly, flatly compressed elements have already been seen in the one-year-old branches on the

periphery of the primary bast, the appearance repeating itself consequently in the bast growth of subsequent years. These bands of flatly compressed cells are afterwards broken into parts which always, and after a time more distinctly, take the form of a bow. By the intercalation of new medullary rays the bast is constantly being divided so that every outer bast part spans two inner. Outside of the sieve parts in the rind are the separated pieces of the ring of sclerenchyma fibres. The pieces are separated by parenchymatous tissue. The ring has been radially broken in consequence of the progressively lateral growth of the cambium, and the adjoining tissue of the rind has pushed itself in. The collenchyma ring also is distributed in parts. Still, there is no essential breaking of it up; rather, in single places, a tangential extension of the cells takes place which then in parting came in and so the parenchymatous tissue masses get their origin. The surface of the stem is covered with the periderm which presents the beautiful alternation of broad zones of wide thin-walled, and narrow zones of small, thick-walled cork cells. As in the pith and medullary rays so in the rind are found scattered crystal masses of calcium oxalate.

The radial longitudinal section shows, in the first place, the wide and narrow vessels, border-pitted, with annular diaphragms; the border-pitted tracheids; the fibre cells, shallow-pitted and with cell contents; the wood-parenchyma cells, likewise with cell contents, with flat pits, shorter, less thickened than the tracheids and joined together in continuous threads. If the medullary rays have been hit, radial lines of their thin-walled cells will be seen running across the section. On the outer border of the wood, we recognize the cambium cells, rich in contents, thin-walled with transverse walls between; then the still active bast, and here, upon the flat cells of the older bast, the

compressed alternating with the uncompressed parts. The laminated periderm shows up very beautifully in the margin of the section. The longitudinal section of this layer is exactly like the transverse section, the cells being of the same breadth or height. By the cutting of the wood the exact course of the medullary rays is apparent to the unaided eye. This comes from the considerable length of the internode within which both the vascular bundles and the medullary rays retain their direction unchanged. The tangential section shows us under the microscope, the medullary rays in the form of broader or narrower stripes more or less parallel to each other separated by corresponding stripes of wood.

As it is not a little difficult always to distinguish the different tissues in the complicated image shown by sections of the wood, we will try another method, viz., that of maceration. For this purpose take a wide test-tube and over some fragments of potassium chlorate pour enough nitric acid to cover the pieces fully. Put into this a not too thin section of the wood and heat to boiling over a flame. Let it stand for some minutes and pour the whole into a larger dish of water. With the glass rod remove the floating section into another dish of water and thence into a drop upon the slide. This experiment should not be made in the same room with the microscope else the gas may damage the instrument. The section should now be disintegrated with needles so as to have its elements separated. If the reagent has properly worked, the middle lamella will be dissolved and the cells will easily come apart. Now we shall find all of the elements, heretofore studied in connection, entirely isolated. They are mostly well preserved, only that they have been robbed of their wood substance and will be stained violet for the most part with chloridide of zinc. First of all we shall see the pit-

ted vessels mostly separated into pieces corresponding to the annular diaphragms.

The tracheïds are especially numerous with attenuated rounded ends and bordered pits. These pits present themselves now in the smaller walls as narrow oblique slits. But by proper focussing it is always possible to demonstrate that they widen outwardly. When some of the tracheïds are found still joined together, the pits appear in the form of a cross, the corresponding pits on the two adjacent cells being inclined in opposite directions. Besides vessels and tracheïds are thin-walled wood-parenchyma cells with large flat pits. They are also recognizable by their compacted and knotty cell contents. We find also isolated forms which are like those of the fibre-cells, occasionally with but one cell cavity but commonly divided into several shorter parts one above the other by transverse or oblique walls. Those with a single cavity are what we have heretofore known as fibre-cells, but which may be better known as "substitute fibre-cells" since they replace the wood-parenchyma cells.

The compound forms which together replace the wood-parenchyma are apparently produced by the division of a single mother-cell. The transverse division walls must have been formed at an early period when the mother-cell was still thin-walled, for they show the same thickness and the same pits as the side walls and must therefore have been thickened at the same time with these.

NOTE.

- (1) See Russow, Bot. Centralbl. Bd. XIII, p. 140.

LESSON X.

STRUCTURE OF THE CONIFEROUS STEMS.

WE shall now undertake the thorough study of the structure of the stems of the fir tree, *Pinus sylvestris*. We shall find the lateral growth entirely different from that of the *Aristolochia*. In the pine the secondary growth of the wood consists entirely of the formation of one element, the tracheïds. Vessels are found in the pine, only in the medullary sheath, in the primary wood of the vascular bundles. A transection shows that the inner edges of the dark-colored wood which projects into the pith consist of narrow elements with somewhat brown walls. A thin longitudinal section shows them to be spiral vessels. Some such vessels, which likewise have bordered pits and spiral bands, constitute a transitional form to the tracheïds with bordered pits only.

We will use alcohol material in making a section of the cambium, the fresh being liable to tear, and the dry difficult to cut. Lay the wood about twenty-four hours in a mixture of equal parts alcohol and glycerine before cutting. Alcohol material has the advantage of having the cell-contents fixed also. The wood should be cut in June or July when the cambium is in full activity and put into alcohol.

Make the section from the periphery of a good sized stem, as the tracheïds in the later annual rings are larger. We will examine the section in glycerine. But in case we use reagents with it we shall previously wash it with wa-

ter. We begin by making a section of the stem from the periphery inward across several of the annual rings, the cambium and the rind.

We see the tracheïds arranged in a radial series and occasionally a row is doubled. These elements are quadrangular; sometimes five- and six-angled. In the fall the walls become thicker and the tracheïd narrower. Immediately adjoining these are the wider and thinner-walled cells of the following spring, thus distinctly marking even to the naked eye the limit of the year's growth. Parallel to the radial rows of tracheïds are the medullary rays, narrow and of one layer of cells, seldom of more, distinguished by their cell-contents of starch. On the radial walls of the tracheïds stand the bordered pits whose structure we already know. Between the tracheïds and the medullary-ray cells are very wide, half-bordered, or one-sided pits, so wide that they cover almost the whole breadth of the wall of the tracheïds. They must be called one-sided because the border is developed only in the tracheïd. The closing membrane is bent forward in the tracheïd and has no torus. Treated with chloriodide of zinc the closing membrane colors blue (1), while it remains uncolored in the two-sided bordered pits. The cells of the medullary rays at those points where they are touched by the tangential walls of the tracheïds are provided with a thickened ledge. (See the medullary ray *m*, Fig. 47, and the tracheïds bearing on it.)

If the section shall strike a zone in which the cells of the medullary rays are empty, we shall find them united to the adjoining tracheïds with two-sided bordered pits. In the immediate neighborhood of the cambium we see the incomplete tracheïds of the young wood. The walls of the cells increase rapidly in thickness toward the cambium zone. In sections from much older stems we see the radial walls within the cambium zone again become

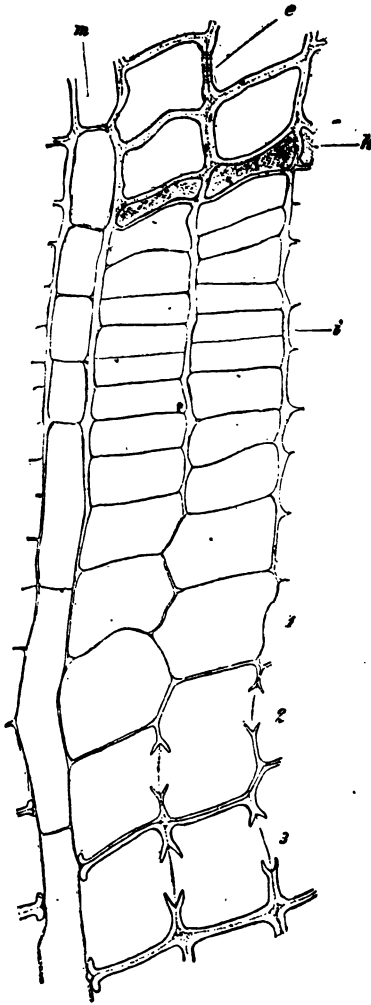


FIG. 46. Part of a transection of an old stem of *Pinus sylvestris*. The section crosses the cambium (*i*, initial layer) and ends on the one side in the young wood and on the other in the young bast. 1, 2, 3, developmental stages of bordered pits; *m*, medullary ray; *e*, sieve plate; *k*, flat cells with brown contents afterwards bearing crystals. $\times 540$.

thicker (2). See Fig. 46. That which we must call the cambium consists of an initial layer theoretically one-cell thick, *i*, which by continuous tangential division furnishes the tissue mother-cells on both the wood and bast sides, and out of these, by the division of the mother-cells, the wood and bast have their origin. No distinct boundary can be drawn between the initial layer and the tissue mother-cells of bast and wood on each side. The youngest partition walls are sharply joined to the radial side walls, *i*. Somewhat older partition walls are, on the contrary, a little thickened at the points of juncture. Upon the wood side the development of the bordered pit may be followed (1, 2, 3). The series of tracheids is continued through the cambium into a row of bast

cells which maintain the radial arrangement quite as fully. The cell walls thicken rapidly on the bast side, but are of a duller white and less sparkling than the wood cells. On the radial walls of the wide bast cells are sieve-plates, corresponding to the places occupied by bordered pits in the wood. In very thin sections they may be recognized by fine pores which penetrate these spots. Mainly, bands of single flattened cells alternate with the thicker layers of sieve-tubes. These bands represent the bast parenchyma. The majority of these cells are indicated by their strongly refractive brown contents, *k*. In cells farther removed from the cambium, one or two crystals may be seen in the brown substance. Since in this tree but one bast parenchyma band is formed in the whole year, these may be used for determining the age of the bast part. Between the cells with crystals lie those filled with starch. So between the sieve-tubes are distributed starch cells and crystal cells, singly or in numbers. Medullary rays continue from the wood through the cambium into the bast, and in the latter a part of its cells contain starch. Only a comparatively narrow zone of the bast will keep the original arrangement taken by the elements. Beyond that zone the radial series is bent, the cell walls begin to be browned, the cell cavity more or less compressed together so that the radial walls appear bent and wavy. Only those cells of the bast and the medullary rays which contain starch are rounded out and full. Finally, the sieve-tubes and crystal-bearing cells are quite compressed and tangentially extended, and like a laminated membrane separate the large starch-bearing cells. The outer rind now seems to consist entirely of the latter cells. Farther towards the outside of the rind one comes to small leaves of cork and from these deeply browned dead tissue is tangentially separated.

The resin-ducts have not been mentioned. Every sec-

tion of the wood shows them. But in the alcohol preparation they have lost their resin contents, and consequently show their structure all the better. In a transection it appears as an intercellular passage, Fig. 47, *i*, surrounded by a layer of large thin-walled cells, *e*. The walls of these cells are browned, have a nucleus, and a wall layer of protoplasm. Bordering on these is a second layer, flatter, and poorer in cell contents, then a more or less perfect, and



FIG. 47. Resin-duct from the wood of *Pinus sylvestris*. *i*, the duct filled with resin; *e*, the cells surrounding the duct; *a*, starch-bearing cells; *t*, tracheids, *m*, medullary ray cells. $\times 240$.

indeed here and there a double layer of large starch-bearing cells, *a*. The latter will be surrounded by tracheids, and will eventually rest against a medullary ray. Conjunction with one such is generally desirable for each resin-duct at some one place. The resin-duct is produced, as their life-history shows, by the drawing apart of certain contiguous cells.

We will now make a section of a fresh stem, and find that the passage is filled with resin. It is very refractive and takes the form of irregularly-shaped drops. Alcohol causes them to disappear. Alcanna tincture colors them as it does oil drops. Upon the section on the slide in a drop of water, lay a thin shaving of the bark of a dry alcanna root. Put on the cover-glass, and add 50% alcohol, and let it stand for an hour. Then remove the alcanna, and it will be found that the resin elements are stained a beautiful, dark red, while the other parts of the section remain colorless.*

*It is much more convenient to use alcanna extract, which may be had of most apothecaries, certainly of all dealers in microscopical goods.—A. B. H.

Chloriodide of zinc colors the tracheid walls of alcohol-material sections yellow-brown; the innermost thickening layer which touches the boundary membrane is, in part, colored violet. Protoplasmic contents and nuclei are easily seen in the imperfectly developed tracheids near the cambium. Thus it is very easy to demonstrate that the fully developed tracheids have lost all their living contents. The cambium, with the youngest adjoining cells, is stained a light violet, the older bast walls a dark violet. The contents of the crystal-bearing cells remain brown, the cells of the periderm red-brown. The thin-walled cells which surround the inner surface of the resin-duct are colored a dull violet.

By staining a section made through the cambium with coralline, we easily observe the gradual extinction of the lignin in the cell walls in the neighborhood of the cambium, the coralline coloring the lignified differently from the un-lignified membrane. Lay the section for some time in coralline soda, and then examine in glycerine. The lignified membranes are colored an intense red, but losing that gradually towards the cambium, the color changes from a red to a pale yellow. The bast has the cell walls a pale, reddish-yellow, the sieve-plates a pronounced rose, especially where they are covered with the callus masses, and the starch grains being stained a rose color bring this tint into prominence in the outer bast.

Now, make a radial section again from the alcohol material, and the tracheids pointed, interlocked, border-pitted, are seen as before. The superficial view of the bordered pit is well known. The bordered pits are small and infrequent in the tracheids formed in the autumn. The medullary rays run across the tracheids. The rays sometimes occur sixteen cells high but are usually much less. They consist (4) of radially-extended, serially-ar-

ranged cells, the middle ones having starch, and on their broad sides, next the tracheïds, showing one-sided bordered pits. The upper and under three rows of cells are empty, with small bordered pits. In this respect they agree somewhat in structure and behavior with tracheïds, and might be so named, but we prefer to limit this term to the elements in the wood part of the vascular bundles. The cambium shows, in the longitudinal view, narrow, elongated cells with end surfaces more or less inclined, and touching each other, out of which the wood and bast proceed, and low broader cells which continue into the medullary rays on both sides.



FIG. 48. *Pinus sylvestris*. Parts of two sieve-tubes with sieve spots. $\times 540$.

In order to examine the sieve-plates (5), we make a radial section again from the alcohol material, and lay it in an aqueous solution of aniline blue (6). After a few minutes, transfer it to glycerine on the slide. The glycerine takes the coloring matter from all of the tissue except the sieves, and makes it impossible to overlook them. The color is a beautiful, durable blue, and the preparation may be made permanent. We can distinguish the sieve-plates in the near vicinity of the cambium, and follow out the same into the region where the sieve-tubes are crushed, and the sieve-plates lose thereby their radial position. Still, before that, the sieve-plates have lost their stainable quality. The sieve-tubes have the form of cambium cells and have the sieve-plates on their radial walls, as the tracheïds have the bordered pits. The sieve-plates are, for the most part, smaller than the bordered pits; they appear as round or oval spots, which are divided into an indefinite number of finely-dotted fields with angular outline, Fig. 48.

At some distance from the cambium, the sieve-plates are covered with the callus-plate, a brilliant blue substance. Farther away these disappear, the sieve-plate is naked and colorless. The sieve-tubes are out of function here. It is not difficult to see that the active sieve-tubes contain protoplasm; still, the nucleus is wanting, most surprisingly, and has disappeared even from the youngest sieve-tube. The crystal-bearing cells of the bast are recognized by their brown contents, are relatively short, meet principally with transverse walls, and probably are produced by the transverse division of the cambium cells. They have numerous prismatic crystals lying near and over each other. Beyond these are the starch-bearing cells. They are shorter than the crystal cells, lie in threads over each other, and are intercalated singly or in a long series between the crystal-bearing cells. They afterwards swell very considerably. The medullary rays may be easily followed from the wood through the bast. They retain their essential structure, only losing the characteristic pitting. The starch-bearing series are always inclosed by a row of empty cells above and below.

The resin-duct in the longitudinal section appears as a long, continuous tube, inclosed by shorter cells with transverse division walls arching more or less into the duct. Sometimes a resin passage is found in a medullary ray. Naturally, it follows a radial course and passes the cambium from the wood to the bast.

Now make a tangential section from the alcohol material. It should be made both in the bast and in the wood. The wood section shows the tracheïds and the severed medullary ray. The latter have a spindle-shaped outline while the cells towards the ends become smaller. The narrowest medullary rays have three cells, the majority

eight; but some have as many as twenty. The narrowest are one layer of cells in thickness, the others may have several layers in the middle, and in that case may have a resin passage in the centre, which will of course be cut across. Make a bast section by cutting away from the outside a number of sections of the old bast till at last we come to the young wood. Examining this section with a low power we inquire first what the still active sieve-tubes contain. We look for the callus-plates, and we easily see

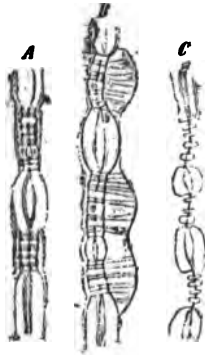


FIG. 49. *Pinus sylvestris*. Walls of sieve-tubes after treatment with chloridide of zinc. *A*, before the formation of the sieve plate; *B*, after the same; *C*, sieve tube which has passed beyond the active stage. $\times 540$.

them, without staining or high magnification, refractive pads lying on the cell walls. Treat the sieve-plate with chloridide of zinc to which a like quantity of potassic iodide of iodine diluted with half water is added. The image of the sieve-plates is in this view much the same as in cross-sections, still the number of them is much greater and so one is more likely to hit upon one favorably situated. We shall find what we are looking for soonest in the edges of the section. The sieve-plate, Fig. 49, *A*, presents itself in profile within the radial walls of the sieve-tube. The walls themselves are somewhat swollen and colored violet by the chloridide of zinc. The sieve-plate is stained a red-brown if it belongs to a still active sieve-tube. This staining comes from the presence of plasma strings which penetrate both sides of the sieve region. The sieve-plates look as though they were traced over with red-brown crayons (see the figure). The callus-plate, *B*, in case the zinc solution is not strong enough to dissolve it, is stained

a red-brown. The sieve-plates of those tubes which have passed their active stage appear a clear violet, *C*. The plasma strings and the callus-plates have both disappeared. Stain the section with aniline blue and examine in glycerine and the luminous blue callus-plates are very clearly seen. We can easily follow the growth of them on one side, and their disappearance on the other.

NOTES.

- (1) Russow, Bot. Centralbl, 1883, Bd. XIII, p. 140.
- (2) Sanio, Jahrb. f. wiss. Bot. Bd. IX, p. 51; E. Strasburger, Zellhäute, p. 39.
- (3) Nach N. J. C. Müller, Jahrb. f. wiss. Bot., Bd. v, p. 398.
- (4) See de Bary, Vergl. Anat. p. 505.
- (5) Janczewski, Mém. de la Soc. d. Sc. nat. de Cherbourg. Vol. XXIII, p. 260; E. Strasburger, Zellhäute, p. 57; Russow, Dorp. naturf. Gesellsch., 17 Feb. 1882, p. 264.
- (6) K. Wilhelm, Beiträge zur Kenntniss des Siebröhrenapparates, 1880, p. 36; Russow, Stzber. d. Dorp. naturf. Gesellsch., 1881, p. 63.

LESSON XI.

STRUCTURE OF LINDEN. BICOLLATERAL VASCULAR BUNDLES OF THE CUCURBITA. SIEVE-TUBES.

A transverse section of a branch of the linden, *Tilia parvifolia*, 5 mm. thick, shows us a large-celled pith whose air-filled cells are grouped rosette-like about single narrower cells filled with fine-grained brown contents. In the outer parts of the pith are gum reservoirs which, being as yet empty, form cavities in the parenchymatous tissue. On the outermost edges the pith is small-celled and the cells filled with finely granular contents. Into this small-celled tissue projects the primary wood part of the vascular bundle. The spiral vessels are seen in the section by their occasional prominent thickened band. We count some five annual rings in a transverse section of 5 mm. in diameter. The spring growth produces large vessels close together which distinctly mark the boundary of the year. Beyond, the wide vessels stand singly or in single groups, and in the last phases of vegetation the cambium forms only narrow cells. Beyond the cambium, the most conspicuous object is the wedge-shaped, pointed bast. In this is a tangentially-arranged lighter and darker stripe. The light stripe is composed of numerous bast fibres solidly united together, whose walls are so thickened as to reduce the cell cavity to a dark point. The stripes have an irregular contour; may even be broken apart. The darker stripes, consisting of narrow, starch-bearing cells, are bast parenchyma and principally rest on the bast fibres. Near the middle of the layer are wide cells, among which we

recognize sieve-tubes. Small cells, apparently cut off from the corners of these, are the conducting-cells. There are about twice as many stripes of secondary bast fibres as there are yearly rings in the wood. Excepting the two first years, two bast fibre layers are regularly produced each year. The outer edge of the figure will have been taken from the primary bast string, which differs in no way from the secondary bast string. The primary medullary rays are mostly two, but sometimes more cell-layers thick, the secondary of but one. They may be traced through the cambium to the primary rind or into the bast. The primary rays are considerably extended and separate the bast parts. The numerous tangential divisions in these medullary rays cause the cells to be tangentially arranged. The outer end of the medullary rays and the primary parts of the bast disappear in the living green primary rind. In the latter and in the extreme ends of the rays are numerous clusters of crystal. Beyond the chlorophyll-containing cells, are the collenchyma cells with their white, thickened corners. The surface of the stem is covered with a regularly developed periderm whose flat cells are the measure of their age, that is, they grow more and more brown toward the outside.

The radial section shows that the vessels of the secondary wood are border-pitted and that between the pits is a spiral band, or inner thickened layer. The vessels are joined at the ends by inclined walls with one large opening. Besides the vessels, especially in the autumn wood, and connected with them by transitional forms, are the tracheïds, thickened like the vessels and with both ends pointed and closed. Between the vessels and the tracheïds are stretched out the wood fibres with small, infrequent, bordered pits and narrow wood-parenchyma cells, filled with oil drops or starch, the longitudinal and transverse walls of which are furnished with unbordered pits. The

wood fibres are longer than the tracheïds, have no *living* contents and bear only water. The pits of the wood fibres open into the cell cavity with a narrow slit which is inclined in a direction opposite to that of the corresponding pit in the adjoining cell. So with a medium adjustment of focus a small cross is seen in the pit. In these wood fibres, as almost universally in the mechanical elements (the stereïds), the stoma-like pits rise toward the left, that is, they follow a left hand spiral line (1). Only where one vessel borders upon another or upon a tracheïd are the pits in the walls large or numerous developed. Those of the walls bordering on the wood fibres are like them sparsely pitted. The pits of the vessels where they touch the walls of the wood parenchyma are bordered only on one side. The autumn-grown wood fibres are very narrow. The medullary rays appear as cross stripes of considerable height in the wood. They consist of right-angular, radially-elongated cells, bearing starch, and particularly in the tangential walls very numerous pitted. In the bast are seen the very long, thick, white, bast fibres; between the strings of bast fibres short parenchyma cells provided with transverse walls and bearing starch and sometimes crystals, and the sieve-tubes, whose sieve-plates when diagonally placed appear to be distributed into several sections by cross bands. Outside of this, the collenchyma and the cork offer something of interest. But since these cells are of equal height and breadth they present the same image in this as in the transverse section.

The tangential section confirms the conclusion drawn from the radial section, of the very considerable extent of the medullary rays. These rays are either of a single layer throughout their whole height or of two layers in the middle. For the rest we find the same as in the radial section.

Turning back now to the transverse section

be able to recognize in this the structure of the wood. The principal mass of the wood is formed of wood fibres, and in the autumn growth they are flatter and occur almost alone. The pits of the fibres are difficult to see, and show but a small border. The vessels and tracheïds are clearly border-pitted, but the pits are very numerous only where these elements touch upon each other. A sharp distinction is with difficulty made between the vessels and the tracheïds in the cross-section. The wood-parenchyma cells are distinguished by their smaller width. They lie chiefly about the vessels, and also distributed singly between the other elements. Their starch contents can be used for their recognition only in thicker parts of the section, because in the thinner places the starch is scattered over the cells by the knife.

Chloriodide of zinc colors the wood part yellow-brown; the cambium, violet. In the bast there is a beautiful alternation between the violet thin-walled parts and the clear yellow thick-walled bast fibres. The elongated medullary rays and the primary rind are violet, the cork red-brown.

Coralline colors the wood cherry-red; the bast-fibres, a strikingly beautiful, brilliant rose-red. The sieve-plates in the transection appear a foxy-red.

On account of the difficulty of studying the secondary wood we will proceed to separate the elements by maceration and examine them separately. We will use *Tilia parvifolia* and proceed as with *Aristolochia* and disintegrate the macerated section with the needles, finding the principal part of the wood to consist of fibres, Fig. 50, *A, B*. The swelling of the walls has much diminished the size of the obliquely-arranged, slit-like pits. The short parenchyma cells are recognized by their contents, either separated or still joined together and resembling

in their outline the wood fibres, among which they lie scattered about. The tracheïds with spiral bands are less numerous and, in contour some, *E*, resemble the wood fibres, others, *D*, the vessels. Finally, we come to the vessels separated into sections as *F*, or forming long tubes. There are long bast fibres

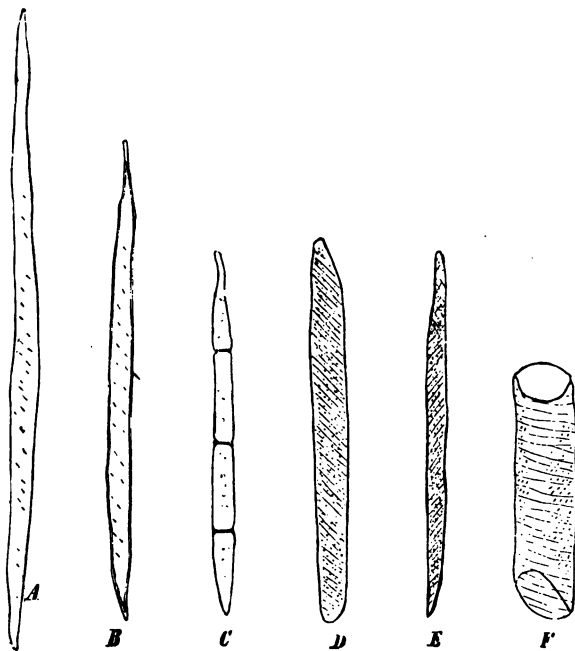


FIG. 50. *Tilia parvifolia*. Cells from the secondary wood and bast isolated by maceration. *A* and *B*, wood fibres; *C*, wood parenchyma; *D* and *E*, tracheïds; *F*, vessels; *G*, bast fibres. $\times 180$.

with very narrow cell cavities, *G*. An attentive examination of the tracheïds and the vessels demonstrates that the slit-like orifices of the pits have an opposite inclination to the spiral bands, in the vessels being much steeper and in the narrow tracheïds

about as steep as they. The tracheïds and the vessels being so much alike it is often difficult to distinguish a wide tracheïd from a narrow vessel, unless indeed the end be perforated and it is not always easy to determine this point. But, in fact, this distinction is of no great account since the two elements pass into each other by minute gradations. So we classify them by their forms and call the tube-like forms vessels and the fibre-like forms tracheïds.

Taking now the *Cucurbita pepo* for examination, we find that the vascular bundle has two bast parts, one on the inside and one on the outside of the wood. These bundles are, therefore, bicollaterally built; the outer bast is separated from the wood by the cambium; the inner immediately joins the wood. To find a full-grown, vascular bundle, take a section of the stem from a point half a metre from the end, where it is some 8 mm. thick. At a point somewhat nearer the growing end, where the stem has a diameter of 5 to 6 mm., the larger vessels are not yet fully developed. Use alcohol material for the investigation. The vascular bundle has no sheath and is not very sharply separated from the surrounding fundamental tissue. The image will be improved by applying aniline blue to the section for a short time and then examining it in glycerine. The portion to which the vascular bundle belongs will take a darker stain than the fundamental tissue. Excluding for the moment the inner sieve part, we find the remainder of the vascular bundle to be so like those already known in the *Ranunculus* and the *Chelidonia* that we should without difficulty class it in the same group. Observing first the transverse section of a fully-developed, vascular bundle, with complete vessels, we look for the most normal case where there are two large vessels; these are among the widest vessels known. Between them lie primary, wood-parenchyma cells, pretty wide, radially

elongated for the most part and walls with net-like thickenings.

Towards the inside are vessels of considerably less diameter than the two described, and farther on are others still smaller. Between these are thin-walled wood parenchyma which continue beyond the bounds of the inmost vessels. The inner bast joins these and is composed of

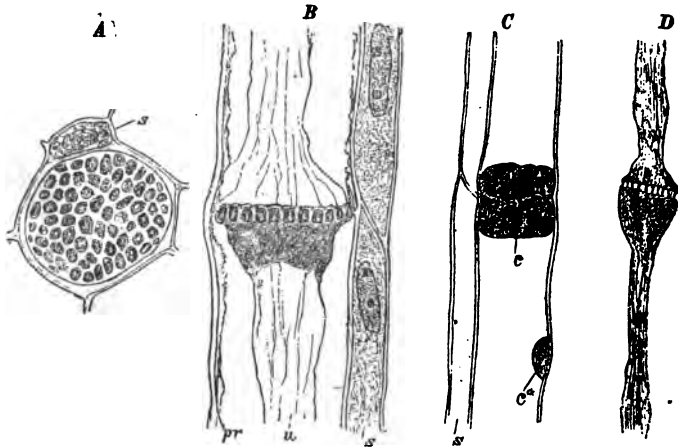


FIG. 51. *Cucurbita pepo*. Parts of sieve-tubes. *A*, transection; *B* to *D*, longitudinal section; *A*, a sieve-plate from above; *B* and *C*, the adjoining parts of two sieve-tubes from the side; *D*, the connecting part of the strings of mucilage from two sieve-tubes after treatment with sulphuric acid; *s*, conducting cells; *u*, mucilage string; *pr*, protoplasmic utricle; *c*, callus-plate; *c**, small lateral callus plate of a lateral sieve spot. $\times 540$.

wide sieve-tubes, narrow conducting-cells and bast-parenchyma. The transversely-placed sieve-plate may be easily seen from above, Fig. 51, *A*. The conducting-cells, *A*, *s*, show very plainly with their contents tinged a dark blue. On the outer side of the wood the thin-walled, radially-arranged, cambium cells follow directly upon the two large vessels and the wood-parenchyma lying between.

Then comes the outer bast which is constructed like the inner. The sieve-plates are easily found in both bast regions, and, according to our magnification appear to be perforated with small or large pores. In the older sieve-tubes, the pores are narrower and divested of strongly refractive substances. So in *A*, Fig. 51, the sieve-plates are often covered with masses of violet-blue matter. In the narrower sieve-tubes on the outer and inner edges of the vascular bundles appear the callus-plates as homogeneous, azure-blue masses. Focussing deep enough, we strike the meshwork of the sieve-plate. Using a low power on a transection, we see that the vascular bundle stands arranged in two rings. The vascular bundles of the outer ring stand before the edge; those of the inner ring alternate with those of the outer. A ring of sclerenchyma fibres, whose elements are of much darker color than the large-celled, fundamental tissue, protects the inner part. Upon these follow, towards the outside, rind-parenchyma containing chlorophyll, and then typically developed, here and there interrupted, uncolored, brilliant-white collenchyma.

At the points of interruption, the rind parenchyma reaches through to the epidermis, which latter bears the stomata at these places. The stem is hollow on the inside. Cross-sections of the stem, where it is not more than 5 or 6 mm. thick, show the large vessels and the cells lying between in the state of formation. It often happens that of the two larger vessels, only one is being formed, the other on the contrary being obliterated; then the one attains an almost colossal diameter. In many cases also, both vessels are obliterated. Finally, there are individual instances in which both vessels occur and both are as large as is usual where there is only one.

Radial sections, rightly taken, show us that the narrowest vessels are the ring and spiral vessels; the widest, the pitted with ring-like, transversely-placed diaphragms. The two largest vessels have walls with irregular, net-like thickenings with numerous pits between the meshes of the net. Sometimes these vessels will be found with entire partition walls, in which case a nucleus and a thin layer of protoplasm on the walls will be found. Many partition walls will be found swollen in the middle in the form of a biconvex lens. Longitudinal sections of the next older parts of the stem will show us in place of these partition walls small rings affixed to the side walls, the nucleus and protoplasmic layer having disappeared. Between the narrow vessels is thin-walled, primary, wood-parenchyma tissue. The cells between the large vessels belong to the thick-walled, primary, wood-parenchyma tissue; they are thickly pitted, even on the partition walls. The walls of these cells which join the vessels perpendicularly are wavy; this causes them to modify the pits of the vessels. In these wood-parenchyma cells are nuclei and a protoplasmic sac.

At both sides of the vascular bundles we may conveniently study the wide sieve-tubes (2), Fig. 51, *B*. Stain the section with aniline blue and examine in glycerine. The latter fluid will withdraw the color somewhat from the cell walls after a little while, but not from the cell contents. Most of the sieve-plates are transversely placed, few inclined. Most of them also are covered with a callus substance, and are correspondingly thickened. See Fig. 51, *C*. Use a comparatively low power. The sieve-plates are colored a pure blue. In the tubes which show the sieve-plates is a sac-like axillary string, *u*. It is a mucilaginous cord widened at the end so as to cover the whole

of the sieve-plate, and colored an indigo blue. The end setting on the sieve-plate is more thickly filled with contents. See in *B*. The collection of cell contents is to be remarked in one or both ends of the sieve-tube; and, if in but one, at the upper end. Besides this, a layer of protoplasm may be found on the walls of the sieve-tubes, *pr*. No nucleus exists. In somewhat younger sieve-tubes the mucilaginous cord may be seen by low magnification pressing through the pores of the sieve-plate into the adjoining sieve-tube.

In each plate the strands are all moving in one direction, but in successive plates they may be going in opposite directions. The phenomenon is not seen in older sieve-tubes, the callus substance having increased on the sieve-plate and narrowed the sieve portion, and through these narrowed pores the slimy contents of the cell continually pass (see *B*) from one to the other. On the outer and inner edges of the vascular bundle the callus-plates cover the sieve-plates, Fig. 51, *C*. They are colored azure blue, and show the sieve-plate in their midst more or less clearly.

The callus-plates consist of two halves which belong respectively to two neighboring sieve-tubes and are connected through the pores in the sieve-plates. A delicate striation is often observable which extends through the connecting pores. See the Figure. When two sieve-tubes are laterally joined, small sieve-spots appear on the common wall and afterwards a one-sided, *c**, or two-sided callus-plate occurs. Conducting-cells, *s*, of the same length as the sieve-tubes, follow their course. They have rich contents and a nucleus. Sieve-tubes, in the process of development, show indigo-blue colored drops of mucilage in their protoplasmic wall-layer. For comparison, it

is necessary to make a longitudinal section of fresh material. The sieve-plates appear as plainly as in the alcohol material. The slimy collections on the sieve-plates are easily seen; but the mucilage nowhere shows itself drawn back from the side walls of the sieve-tubes in the form of a string. This appearance is due to the influence of the alcohol.

NOTES.

(1) See Schwendener, *Das mech. Princip.*, p. 8.

(2) For this compare principally de Bary, *Vergl. Anatom.*, p. 179; K. Wilhelm, *Beiträge zur Kenntniss des Siebröhren-Apparates dicotyle Pflanzen*; E. v. Janczewski, *Etudes comparées sur les tubes cribreux*, *Mém. de la soc. nat. des sc. nat. de Cherbourg*, T. xxiii; Russow, *Stzber., der Dorp. naturf. Gesellsch.*, Jahrg. 1881, u. 1882.

LESSON XII.

VASCULAR BUNDLES OF THE AXILE CYLINDER, AND THE SECONDARY LATERAL GROWTH OF THE ROOTS.

FOR the study of the axile, vascular-bundle cylinder of the roots, we will take a root of the common onion, *Allium cepa*. One may have plenty of material at any

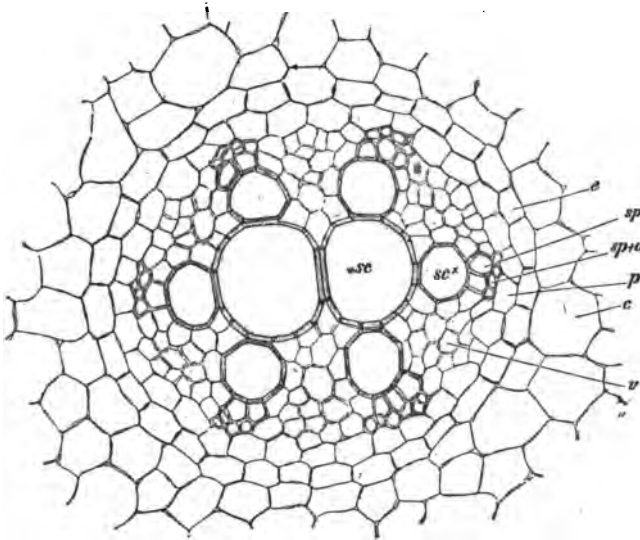


FIG. 52. Transection from the base of a large adventive root of *Allium cepa*. c, rind; e, endoderm; p, pericambium; sp+a, ring vessel; sp, spiral vessel; sc and sc^x, scaliform vessels; v, bast. $\times 240$.

time by putting an onion in a glass of water and letting the roots sprout. Fig. 52 is a section of such a root near the base. The epidermis and the very stout rind tissue are left out of the drawing. Still one may see some of the cells

bordering the endoderm at *c*. The endoderm, *e*, shows in a characteristic manner, dark shadows on its radial walls. These shadows are caused by the wavy bending of the middle part of the walls. Such an endoderm always consists of one layer. We have already met it in the envelope of the vascular bundles of the leaf of *Iris*, which shows it to be not confined to the roots. In the middle of the vascular-bundle cylinder there are, in this case, two wide, scaliform vessels, *sc*. In other cases more or less than two may be seen. If the root is not old enough, the central and perhaps adjoining vessels will be thin-walled, not fully developed. Adjoining the central vessel or vessels are almost always six narrower scaliform vessels, *sc**, and upon the latter follows a group of quite narrow spiral and ring vessels, *sp*, *sp+a*. The size of the vessels diminishes from the centre outward and the outermost are the ring and spiral vessels. Herewith the root differs from the stem. The wood occupies about 180° of the circumference of the cylinder, being arranged in the form of a six-rayed star. The bast, *v*, alternates with the wood. This is the general law with respect to the axile, cylindrical, vascular bundle of the roots. A layer of parenchymatous fundamental tissue laterally separates the wood from the bast. The latter is recognized by the white sparkling walls of its cells. It consists of sieve-tubes and conducting-cells not distinguishable with certainty in a cross-section. The vessels and the bast are separated from the endoderm by a single layer of pericambium cells. Concentrated sulphuric acid will dissolve the whole section with the exception of the epidermis and the cell-layer bordering upon it, and the vessels and the endoderm. The latter will be colored a beautiful yellow. The endoderm, which will indeed in part bend about during the action of the acid, shows the middle band in its ra-

dial walls beautifully undulated. A similar appearance is observable on the outer layer of rind cells bordering on the epidermis. The cells in question are bound fast together under each other and form a sort of outer endoderm which is sometimes known as the epidermoidal layer(2). A longitudinal section shows us the vessels already mentioned; and staining with coralline, the sieve-tubes and sieve-plates colored rose-red are easily made visible. The conducting-cells, shorter and filled with contents, are easily distinguished. The

undulation of the middle band of the radial walls of the endoderm cells, looked at from the surface, appear like a scaliform thickening. The pericambium cells have the same form as the endoderm cells, yet of greater length. The inner endoderm takes the color (coralline)

with some avidity, while the outer endoderm is contrasted from the surrounding tissue by its colorlessness.

We will take for further study a section of the root of *Acorus calamus*, sweet flag, shown in Fig. 53. The vascular rays do not meet in the centre of the cylinder, but are arranged in eight segments of a circle, while the middle is filled with medullary tissue. The larger vessels lie nearest the centre, the smaller toward the periphery. The bast, *v*, alternates with the vascular rays. The two are laterally separated by a single or double layer of paren-

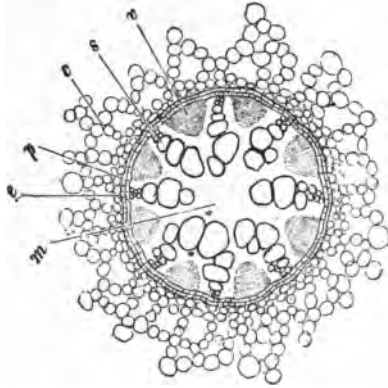


FIG. 53. Transection through the root of *Acorus calamus*. *m*, pith; *s*, wood; *v*, bast; *p*, pericambium; *e*, endoderm; *r*, rind. $\times 90$.

chymatous fundamental tissue, and from the endoderm, *e*, by a single layer of pericambium. The endoderm consists of flat thin-walled cells, and it and the pericambium and all the remaining fundamental tissues of the vascular-bundle cylinder are filled with starch, which makes the starchless bast appear very distinct in the image. The cells of the inner rind are separated into single-celled layers by numerous air passages. In the periphery the rind cells are compacted together into a solid layer, several cells thick. The outer hypodermal rind layer consists of radially-elongated cells and forms in this, as in other roots, an outer endoderm which persists, while the epidermis dies and disintegrates. Add potash lye, and dissolve the starch, and the dark shadows in the radial walls of the endoderm are distinctly seen. Treat with sulphuric acid, and we see that the whole cell-wall of the outer endoderm is cuticularized, but only the shadow-forming band of the inner endoderm. The cells of the outer endoderm contain resin. There is a mechanical significance to these endoderms. They protect both the surface and the axile vascular-bundle cylinder. By the suberization of their cell-walls they attain great solidity and little extensibility. But the interpassage of fluids between the vascular-bundle cylinder and the rind is not thereby interfered with, since the cells of the inner endoderm are suberized only or principally on their radial walls (3).

A cross-section of a root of *Iris florentina* shows us an axile vascular-bundle cylinder, quite exactly like that of the *Acorus*, except that the endoderm is differently built. See Fig. 54. The cells are unilaterally thickened, U-shaped and the thickening beautifully laminated, *e*. Exactly in front of the vascular ray is a single unthickened cell, *f*. It is known as a transit-cell (4), and being permeable maintains the connection with the surrounding rind, *c*.

The thickened layer swells and dissolves in concentrated sulphuric acid, the cuticularized middle lamella only remaining and forming a delicate envelope about the endoderm and transit cells. The middle lamella between the vessels and in the pith is not dissolved, but forms a delicate, yellowish-brown network. A tangential section, which just grazes the endoderm, teaches us that the longitudinal stripe, which lies in front of the wood parts, consists of long, thickened, alternating with short, unthickened, transit cells, full of cell contents. Sometimes, two short transit cells follow each other.

The roots of dicotyledons are less favorable for study than those of the monocotyledons, but, having become acquainted with the latter, we shall have no difficulty with the former. Make a cross-section from the base of an adventive root of a runner of *Ranunculus repens*.

The axile vascular-bundle cylinder is not so sharply differentiated from the rind tissue as in the monocotyledons, but by attentive examination we shall find on the border of the two the dark shadows which mark the endoderm. The axile cylinder is divided into four or five vascular rays, according to the size of the root. The larger vessels lie here towards the inside and the smaller towards the outside. In monocotyledons, the innermost vessels are distinguished by their large size. This is rarely seen in the dicotyledons and not at all in the *Ranunculus*. The vascular rays extend to the middle of the cylinder, and abut more or less

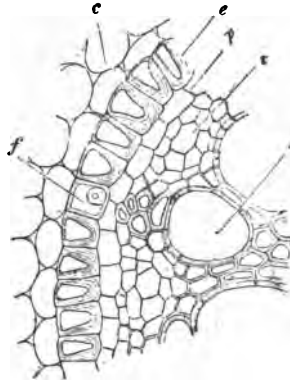


FIG. 54. Part of a transection through the root of *Iris florentina*. e, endoderm; p, pericambium; f, transit cell; r, bast; s, vessels in the wood; c, rind. $\times 240$.

fully against each other. The innermost vessels are latest in developing, and remain in the condition of thin-walled elongated cells. The bast alternates in the ordinary way with the wood.

The roots of the vascular cryptogams are simpler, and yet are constructed on the same type as those of the phanerogams.

Take next a rootlet of *Taxus baccata*, about 1 mm. thick, and make a cross-section. The rind consists of about ten thicknesses of parenchyma cells. The outer cell layer of the rind is not especially differentiated, there being no distinct epidermis. The inside of the section is filled with the axile vascular-bundle cylinder, which is surrounded by an endoderm. The latter consists of flat, thin-walled, suberized cells, whose walls are browned, and whose diameter is considerably less than that of the rind cells, the radial walls being characteristically shaded. A single-celled, thickened layer is developed about the endoderm. The cells are of the same width as those of the rest of the rind, but the radial walls are furnished with a thick, bright, yellow ring. These ring-like thickenings correspond to that in the neighboring cells, which give them in section the form of a biconvex-lens. The axile vascular-bundle cylinder shows a double-arched wood body, extending across it, at the opposite ends of which is a narrow, spiral vessel. Inward, and joining these, is a strip of tracheids with bordered pits, characteristic of the conifers. They are easily recognized by their clear yellow, strongly-thickened walls. These tracheids almost always meet in the middle of the cylinder, forming a plate. On each side of the tracheids, lies a strip of narrow, thin-walled, fundamental-tissue cells, bearing starch. Upon these, borders a somewhat small-celled tissue of thin-walled bast. Finally, beyond this, a large-celled starch-bearing layer, about

four cells thick. The latter are joined together, making a complete circle, somewhat reduced before the spiral vessels. They represent the pericambium.

If now we examine a cross-section about 1.3 mm. in diameter, we shall find the two sides of the plate of tracheïds dividing and becoming transformed into cambium, which produces tracheïds on the inside and bast on the outside, and on both sides medullary rays. Now examine

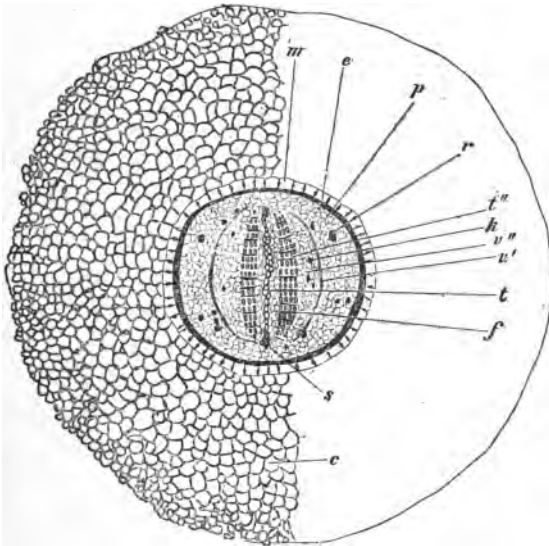


FIG. 55. Transection of root of *Taxus baccata* after the beginning of the lateral growth. c, rind; m, thickening layer; e, endoderm; p, pericambium; s, spiral vessels; t, primary tracheïd plate; f, stripe of fundamental tissue; t'', secondary tracheïds with medullary rays; v'', secondary bast; v', compressed primary bast; k, cells in secondary bast with crystals in the walls; r, resin-bearing cells in pericambium. X 42.

the further activity of the cambium in a section of a root 2 mm. wide, as shown in Fig. 55. It shows the already well-known relations: the rind, c, which has lost its hairs from the outer layer of cells; the outer strengthening layer, m; the endoderm, e; and the axile cylinder. The

outer cell-layer of the pericambium has in the meanwhile begun to divide and has been transformed into a few layers of periderm. On both sides of the tracheid plate we see the inner inactive layer of fundamental tissue, *f*, the so-called connective tissue: beyond, the newly-formed radially-arranged tracheids, *f''*, with numerous intercalated medullary rays. These relations are more easily seen if one adds a little potash lye to the preparation. The vessels, *s*, on the edges of the middle plate come out distinct and black.

The tracheid plate, *f'*, as well as the tracheids formed by the cambium, *f''*, is colored a beautiful yellow. The connective tissue remains white. The secondary wood layers have a plano-convex outline which runs to a point at the edges but not here in front of the vessels. On the outer side of the wood body we find the cambium and outside of this the secondary bast, *v''*, which after treatment with the potash appears white but in which single cells are black, *k*, having crystals of oxalate of lime in their walls. The primary bast forms a layer of compressed cells on the outside of the secondary. The potash brings out the pericambium more distinctly than before, also the resin-bearing cells with their yellow-brown contents. The cork layer produced from the pericambium is colored a yellow-green, the thickening ring of the strengthening layer a bright yellow. The endoderm is compressed by the cork layer.

Making a section now of a root 2 mm. thick which has thrown off its rind and shows a dark brown surface, we find the section has a fully closed wood part, and makes an image indistinguishable from that of a branch of like size, were it not that here the place of the pith is occupied by the primary tracheid plate.

The vessels at the edges of this plate are somewhat difficult to make out. The plate is inclosed by the starch-

bearing connective tissue which here to a certain extent displaces the medullary crown and into which the oldest medullary rays open. The two wood bodies have united in front of the vascular groups and the medullary rays at that place are scarcely noticeable by special width. The surface receives the inclosing cork layer produced from the outermost pericambium layer. The secondary rind consists of secondary bast and the elongated medullary rays. That which represents the primary rind here will consist of the enlarged and numerically increased pericambium cells closely packed with starch.

Longitudinal sections show that the middle tracheid plate consists of the same elements as the secondary wood. We find the spiral vessels on the edges of the plate, and observe that the cells of the endoderm are quite short while those of the strengthening layer are far larger and are even longer than the adjoining cells of the rind. Coralline stains the tracheids a beautiful coral-red and brings out the sieve-plates in the primary and secondary bast. The rings of the strengthening layer also absorb the coralline.

NOTES.

(1) De Bary, *Vergl. Anat.*, p. 365, where the older literature will be found; Olivier, *Ann. d. Sc. nat. Bot.*, VI Ser., XI Bd., p. 5, ff.

(2) See v. Höhnel, *Stzber. d. k. Ak. d. Wiss. in Wien, math. naturwiss. Cl. Bd. LXXVI, I Abth.* 1877, p. 642; Olivier, l.c.

(3) Schwendener, *Abh. d. kgl. Ak. d. Wiss. in Berlin*, 1882. The protective sheath and its strengthening.

(4) See last work quoted, p. 13.

LESSON XIII.

VASCULAR BUNDLES OF THE FERNS AND LYCOPODS.

IN the leaves and stems of the ferns the vascular bundles are concentrically built, whereby the wood is almost or quite fully surrounded by the bast.

Make a section of *Pteris aquilina*, in which it is possible to get a good knowledge of the vascular bundle, even when the numerous, sclerenchyma strings in the fundamental tissue do not permit us to make a good section. Make the section from the rhizome directly behind the growing point or through the petiole of a young leaf. The vascular bundle will be sufficiently developed, while the fundamental tissue will not be much hardened. The bundle will be the same in the rhizome and the petiole, and a cross-section of it from the base of the latter is shown in Fig. 56. Choose a small bundle. We first notice the large, border-pitted, scaliform vessels, *sc*; still, the smaller vessels are thickened also and only the few on the two ends of the wood which adjoin the protoxylem elements have spiral thickenings, *sp*. The vessels are surrounded, when they do not touch each other, by starch-bearing, wood-parenchyma cells, *lp*. Wood-parenchyma and vessels form the wood part which is almost perfectly enclosed by the bast. The latter borders on the wood parenchyma with sieve-tubes, *v*, which are succeeded outwardly by narrow conducting-cells, *s*, which are filled with protoplasm—not starch, as iodine will show. But single, starch-bearing cells are sparsely distributed through this tissue.

The periphery of the bast takes on a layer of still narrower, thick-walled protophloëm elements. The bast is surrounded by a simple layer of cells, *pp*, filled with starch, which in its position, but not in its origin, resembles pericambium and may be called periphloëm. Around this preliminary sheath is the endoderm, *e*, thin-walled, free from starch and suberized, and showing the dark

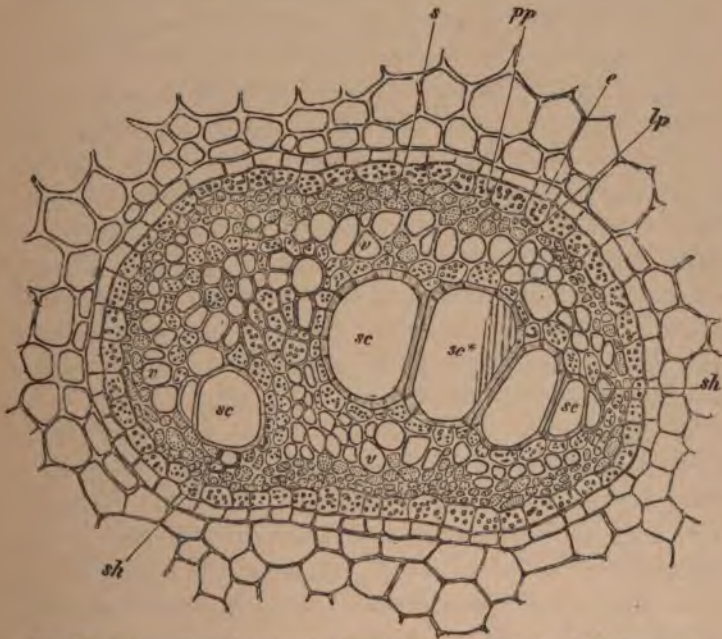


FIG. 56. Transection of a vascular bundle from the petiole of *Pteris aquilina*. *sc*, scaliform vessels; *sp*, spiral vessels. In *sc** a piece of the scaliform perforated wall is seen; *lp*, wood parenchyma; *v*, sieve tubes; *s*, conducting-cells; *pr*, protophloëm; *pp*, periphloëm; *e*, endoderm. $\times 240$.

shadows on the radial walls. The periphloëm and endoderm cells correspond to one another and suggest a common origin in the same mother-cell. The wood at its two edges, together with the covering of wood-parenchyma, borders directly on the periphloëm or on the proto-

phloëm. At these two points, the bast is either wholly or nearly interrupted, but in other ferns this break may not occur. The walls of the endoderm cells are often broken by the cutting and then the vascular bundle will be separated from the fundamental tissue. The cells of this tissue, bordering on the endoderm, are sometimes much thickened and then are colored a yellow-brown. The cross-section through the rhizome shows a browned and cuticularized parenchymatous tissue under the deep brown epidermis which, further towards the inside, is colorless and full of starch. This starch-bearing, fundamental tissue is penetrated with the vascular bundles and the red-brown sclerenchyma fibres; the latter form plates which run between the vascular bundles more or less parallel to them. The outer bundles are in immediate contact with the endoderm on the outside, supported by such sclerenchyma fibres, which here represent the mechanical tissue. In the inside of the bast the relations are similar, there being a hypodermal ring of red-brown sclerenchyma fibres which rest on the epidermis.

A longitudinal section shows us all the wide, scali-form vessels again. The ends are much inclined, ladder-like, border-pitted and in part perforated (1). On the side walls separating the two vessels, it is easy to see that the pits are bordered on both sides, and the closing membrane has a thickened torus; but on the walls bordering on the wood-parenchyma cells the pits are bordered only on one side, and the closing membrane has no torus. The section may also hit one or the other of the spiral vessels, and the plates of the sieve-tubes may also be discovered, but only by the most careful examination; the latter may be found much more easily by the help of coralline staining, which also will show the sieve-plates much inclined and parted into numerous fields by thickened bands; besides these, the lateral walls of the sieve-tubes bear sieve-

spots. Together with the sieve-tubes, we find the slender, conducting-cells with finely, granular contents and nucleus, and in contact with the vessels the starch-bearing, relatively-short, wood-parenchyma cells. Resembling the latter, are the starch-bearing cells of the periphloëm. Small pores are seen in the walls of the long-pointed, sclerenchyma fibres of the fundamental tissue.

It will be interesting to make a transection of the petiole of *Polypodium vulgare*. The vascular bundles are very thickly sheathed about, but the sheath corresponds not to the endoderm but to a strengthening layer. This layer is but a single cell thick and these cells are thickened only on the inside walls and are there colored a dark brown. The essential endoderm lies immediately within the strengthening layer and is scarcely recognizable on account of its cells being flattened down by the pressure of this layer. Next within comes the starch-bearing, single stratum of periphloëm cells, then the bast tissue consisting of cells, of almost the same width. The conducting-cells are distinguished by their contents and, as is apparent, are mingled with the sieve-tubes. The closely grouped vessels are surrounded without by a single layer of starch-bearing wood-parenchyma cells which at the two small edges of the wood-part may extend even to the periphloëm.

Prepare now a cross-section of the petiole of *Scolopendrium vulgare*, where we shall find the two vascular bundles reduced to one. Two wood portions lie apparently in one vascular bundle; rather, in a compound bundle, either near each other, or, as is frequently to be seen, united at one point so as to form an X-like figure. The stouter legs of the figure are turned towards the upper side of the petiole. The small vessels are found at the ends of the legs. From the ends of the upper legs small vascular bundles are often seen branching out. The cells of the bast are all of uniform size, but the conducting-cells mingled

with the sieve-tubes are easily recognizable by their contents. On the surface of the figure, the periphloëm appears several layers thick and with somewhat thickened walls. The outer circumference of the compound bundle is deeply fluted at three points, viz., above and at the two sides; and here follows, on the endoderm, a plate of sclerenchyma fibres, red-brown, and thickened almost to the extinction of their cell cavities. Higher up in the leaf, the wood part gradually assumes the form of a T. The three

strengthening sclerenchyma strings are always present even though reduced.

In the *Lycopodium* species the axile vascular-bundle cylinder appears in a relatively more highly complicated form. Still the relations of the parts should not be difficult to make out after having

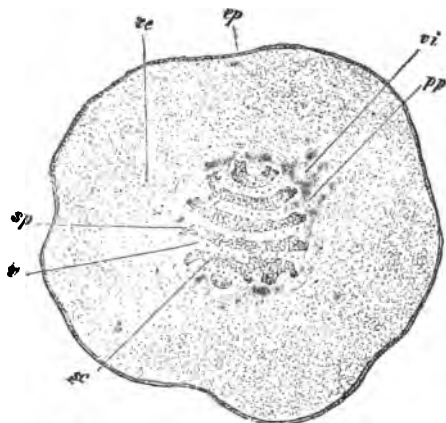


FIG. 57. Transection of stem of *Lycopodium complanatum*. *ep*, epidermis; *ve*, outer sheath; *vi*, inner sheath; *pp*, periphloëm; *sc*, scaliform vessels; *sp*, ring and spiral vessels; *v*, sieve elements. $\times 26$.

seen the compound vascular-bundle in the petiole of the *Scolopendrium*. We have in fact to deal with an axile vascular-bundle cylinder, in the *Lycopodium*, which is formed by the blending of several vascular bundles built like that of the last example. We will take the *Lycopodium complanata*, but another species will do as well. Color the section with an aqueous solution of safranin. Fig. 57 represents what we shall see. First we have the epidermis, *ep*; then the rind cells which at first have wide cavities but which toward the inside diminish in width, and

increase in thickness of wall till they form an almost solid sclerenchyma sheath which we will call the outer sheath, *ve.* Between these strongly thickened rind elements are air-filled, intercellular spaces. The outer cells of the rind are colored by the safranin a cherry-red, the inner thickened cells a rose-red. The thickened rind cells suddenly cease and there follow two or three layers of tangentially-elongated-polygonal cells colored a cherry-red. These cells form the endoderm, but are distributed in several layers, have no undulating bands and are not otherwise characteristically thickened. But on the other hand they are, like the cells of the endoderm, cuticularized and withstand the action of sulphuric acid very well. We will designate this envelope of cells, *vi.*, the inner sheath. Next follow several layers of likewise wide cells, which often contain starch, and have white glittering walls appearing as if swollen. By long staining they are colored an orange-red. These cells take the place of the pericambium and may therefore as in the ferns be called periphloëm, *pp.* Now we come to the beautifully stained cherry-red xylem layer. It consists of wide scaliform vessels, *sc.*, separated by no intervening cells, and at the thin edges of protoxylem elements, that is, narrow, ring and spiral vessels, *sp.* In this species the wood layers run across the cylinder and more or less parallel to each other. They are somewhat concave on one side and convex on the other, and one can see by reference to the stem in its procumbent position that those stripes which were parallel to the surface of the ground had their concave sides turned upward. The small vascular bundles of the leaves, when they enter the central cylinder, are joined, as in the ferns, to the group of spiral vessels of a wood layer. The wood stripes often anastomose as in the lower ones of the illustration. In the elongated stem of the *Lycopodium selago*

all the wood stripes are united and form a star. These wood elements are surrounded by a single layer of thin-walled narrow cells which we will designate the wood parenchyma, as in the ferns. On the ends, they extend with their protoxylem and wood-parenchyma to the protophloëm tissue. Between the wood bands is the bast, the larger cells being the sieve-tubes, *v.* The cells are white, with strongly refractive walls, narrow, the middle row only being somewhat wider. In a good staining, the walls of the sieve-tubes are colored a rose-red, while the rest of the bast is colorless. On the edges of these bands of sieve-tubes are the narrow protophloëm elements. The sieve-tubes reach the periphloëm, by these protophloëm cells, the essentially wider cells of the former being clearly distinguished from the wood and bast. In cutting the section, the inner bast and wood elements of the vascular-bundle cylinder are easily separated from the rest of the tissue at the inner edge of the protophloëm.

The longitudinal section shows us first the epidermis and next the rind cells running diagonally towards it. Next the sclerenchyma fibres of the outer sheath; and then the elongated parenchyma of the inner sheath; the periphloëm with white thick walls and diagonally-placed partition walls; the scaliform vessels and the narrow, for the most part much-extended ring and spiral vessels, and finally the bast. The latter consists of very long cells which join each other at the ends with more or less diagonally-placed walls. By the help of coralline or aniline blue, we may recognize the small inclined sieve-plates. Only the wide cells in the bast are sieve-tubes; the more numerous, narrow cells with sparkling granular contents are conducting-cells.

NOTES.

- (1) See De Bary, Comparative Anatomy, p. 170.

LESSON XIV.

CORK. LENTICELS.

WE have already had an opportunity in various objects to learn something of the nature and structure of cork. Nevertheless, we will now direct our particular attention to this object and learn to know the lenticels on the one side, and, on the other, the cork cell walls and their reactions.

A cross-section through a branch of *Sambucus nigra*, about 3 mm. thick, shows us the vascular bundles distributed in the medullary crown, about the wide, large-celled pith, already united by interfascicular cambium. The cambium has already begun its activity, and is now forming in the usual way, secondary bast without, and secondary wood within, both in the vascular bundles and in the interfascicular spaces. The primary bast is supported on the outside by sclerenchyma fibres. The rind is from ten to fifteen cells thick. The projecting edges of the stem have a thick, hypodermal, collenchyma layer, which in the furrows is reduced to a layer three or four cells thick. The collenchyma layer is perforated at the stomata by the green, parenchyma cells of the rind penetrating through to the epidermis. In a stem, 4 mm. in diameter, the cork formation has already begun by the tangential division of the collenchyma cells immediately bordering on the epidermis. The inner of the two sister cells again divides, and the middle cell thus formed, subsequently acts as the cork cambium cell. This is easily made out after the periderm has

become several cells thick, Fig. 58, *ph*. The outermost, in each series of cells, is the outer part, and the innermost the inner part, of the original collenchyma cell, *cl*; the flat cell next to the inner part, *ph*, is the cork-cambium or phellogenetic cell. In a favorable section, one may see a curious incident in the formation of a continuous cork layer which begins under the stomata. The primary rind cells which surround the breathing cavity begin to divide, and the

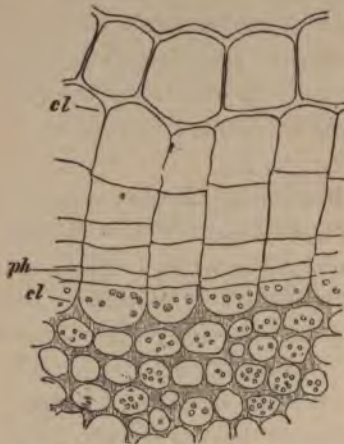


FIG. 58. Transection through the upper surface of a young stem of *Sambucus nigra* epidermis. *ph*, phellogen; *cl* and *cl*, the upper and under parts of the original collenchyma cells. $\times 240$.

division reaches over into the adjoining collenchyma cells. Soon, under the stoma, a meniscus-shaped layer of dividing cells is formed, Fig. 59, *pl*, which produce towards the surface colorless oblong cells, *l*, and towards the inside cork-rind cells, *pd* (phellogenetic cells). The upper are designated "filling" cells. They take a brown color, but are not suberized, and by their numerical increase so press upon the epidermis as to rupture it. Thus are the rind pores or lenticels

produced. Examined with the naked eye, the lenticels appear to be furrows surrounded by two lip-like pads. The brown color of the filling cells is quite apparent. On the younger places of the stem, the lenticels appear as oblong swollen spots. Still younger stages are indicated by a somewhat brighter color. The section should be made through these places in order to show the earliest stages of development. Directly after the rupturing of

the epidermis, the division of the collenchyma cells begins, which leads to the formation of the periderm. The filling cells of the lenticels separate from each other; but, as they disorganize from without, the cambium builds them up from beneath. The spaces between the filling cells are filled with air, and thus the atmospheric air is admitted to the inner tissue of the stem. It thus takes the place of the stomata in those older parts of the stem in which cork has begun. For the winter, somewhat closer formation and more resistant filling cells are formed.

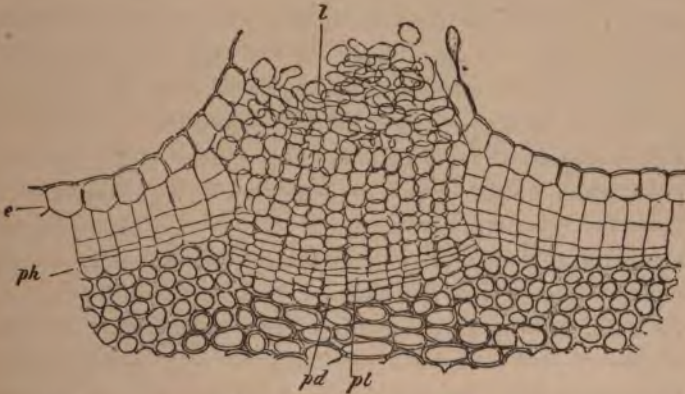


FIG. 59. Transection of a lenticel of *Sambucus nigra*. *e*, epidermis; *ph*, phellogen; *z*, filling cells; *pl*, cambium of lenticel; *pd*, phelloderm. $\times 90$.

An essential enveloping layer for the winter time formed of narrow cells, which touch each other, is not found in *Sambucus*, as in many other plants. But the intermediate layers which are intercalated between the filling cells from time to time serve the same purpose. The cells of these closing and interstitial layers are suberized, but have intercellular spaces between them, so that the closure is not quite perfect (2). In old stems of *Sambucus*, the periderm is ruptured longitudinally. These clefts go through

the lenticels without injuring them. The latter are preserved still in quite old stems, while the outer periderm layers are interleaved between them.

We will next take the *Cytisus laburnum* for the study of the structure of cork cells as they are remarkably thickened in this species. A cross-section through the rind of an old stem shows the periderm constructed of but one kind of cork cells regularly arranged in a radial series. The youngest cork cells are colorless, the older yellow, and the oldest a yellow-brown. The outside cells are tangentially elongated even to the closing up of the cell cavity. All these cork cells are much thickened, especially on the outside. Without the help of reagents, the delicate middle lamella is easily distinguished, also a stout clearly unlaminate, secondary, thickening layer, and on the inside of the latter a tertiary thickening layer. So each wall, which separates any two cells, consists of at least five layers: the middle lamella which here represents the primary wall and is lignified; the two secondary thickening layers which are alone suberized; and the two tertiary thickening layers, which often betray their cellulose character and may be designated the cellulose layer, but in this case are a little lignified. Chloriodide of zinc colors the cork cells yellow to yellow-brown, the younger darker than the older, the tertiary layers the darkest. Characteristic reactions on cork substance or suberine are those of potash, the maceration mixture and chromic acid (3). Treat the section with potash lye and notice that the cork cells become yellow. Careful warming on the slide under the cover-glass increases the intensity of the color. With the maceration mixture (chloric acid, potash and nitric acid) we get the ceric acid reaction. The cold mixture brings out all the parts of the cork cell in a yellow-brown color. Heat the preparation with an added quantity

of the reagent, when necessary, and the whole section will be dissolved except the suberized membrane. The colorless globular masses which remain are the so-called ceric acid, soluble in alcohol, but much more easily in ether. Strong chromic acid dissolves the whole section except the suberized layer of the cork cells, and after a while this becomes so transparent as to be difficult to find. Still it does not disappear. Although the middle lamella has been dissolved, the secondary thickening layers still hang together.

The common flask cork (from *Quercus suber*) consists of almost cubical, thin-walled, relatively large cells, which are commonly somewhat thicker and flatter near the limit of the year's production, and are succeeded by the cubical cells again. Potash colors the section yellow especially the thick-walled cells. The five layers of the double walls between the cells are traceable as in the last specimen. The tertiary layer does not give the cellulose reaction at first, but only after proper treatment. The suberine reactions are even more beautiful than in *Cytisus* especially the ceric acid reaction.

It often happens that from the phellogen not alone centrifugal cork cells, but also centripetal rind cells, are formed, the so-called "phelloderm."

The phelloderm seldom reaches the thickness that it has in the *Ribes* species. Prepare a cross-section through an old stem of *Ribes rubrum*, and we shall find under the thin-walled, brown cork-layer, first, the phellogen and then a thick layer of flat rind cells containing chlorophyll; the latter are arranged in radial rows which coincide with those of the adjoining cork. In the inner parts of the phelloderm, the radial arrangement is lost in consequence of the supplementary extension. The innermost phelloderm cells adjoin the collenchyma of the rind.

All those formations which arise from the phellogen are included in the term periderm. In *Ribes*, therefore, the periderm is formed of cork (phellem) and cork rind (phelloderm). It is interesting to make a section through a this year's stem of *Ribes rubrum*, in which the cork formation has but just begun. We shall see the first beginnings of the formation of the phelloderm, and perhaps, demonstrate that in this plant the phellogen is pretty deeply embedded in the rind. The extreme outside portions, being cut off from the sap-bearing tissue by the cork layer, soon die, become brown and are thrown off as the so-called bark.

NOTES.

- (1) Literature in de Bary, *Vergl. Anat.* p. 560; v. Höhnel, *Stzber. d. math. naturw. Cl. d. k. Ak. d. W. in Wien*, Bd. LXXVI, 1877.
- (2) Klebahn, *Jen. Zeitschr. f. Naturw.*, Bd. XVII.
- (3) Introduced by v. Höhnel. *Work and Vol.* quoted above, p. 522.

LESSON XV.

STRUCTURE OF THE FOLIAGE AND FLORAL LEAVES. THE ENDS OF THE VASCULAR BUNDLES.

WE shall now take a series of objects which will make us acquainted with the structure of leaves. We shall begin with the foliage leaves and take those forms which will show us the widest possible differences in the inner structure of the leaves. The first example shall be *Ruta graveolens*.

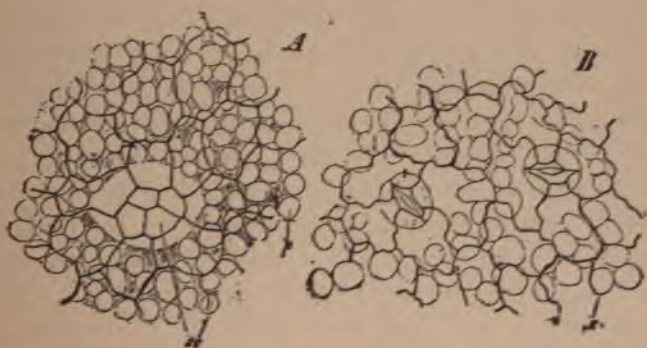


FIG. 60. Leaf epidermis and adjoining tissue of *Ruta graveolens*. *A*, epidermis of the upper side; *sc*, epidermis cells over a secretory receptacle; *pa*, palisade parenchyma; *B*, epidermis of the under side; *s*, sponge-parenchyma. In *A*, the are filled spaces are shaded; in *B* they are clear.

colens whose leaves are mostly retained through the winter. The leaves are doubly pinnated, the leaflets a reverted ovate. Held towards the light clear points are seen in the leaf. They are reservoirs of essential oil; "inner glands" in the tissue of the leaf. We make first a superficial section of the epidermis and observe first that the upper side, Fig. 60, *A*, has but few if any stomata, while

there are many on the under side, Fig. 60, *B*. Both on the upper and under side four epidermal cells lie over the inner gland, *A*, *sc*, somewhat depressed in the middle. In thicker parts of the section, where the reservoir has not been cut by the knife, one may find a yellow strongly refractive drop of matter. By deeper focussing one may see that the tissue of the upper side of the leaf consists of cells whose optical section is round, *A*, *p*. These cells are laterally almost entirely separated from each other and the intercellular spaces filled with air. On the epidermis of the under side, cells with a like round optical section are seen but in much smaller number, *B*, *s*. These cells also are separated with air, and free wide breathing cavities are seen under the stomata *B*. Now make a transverse section perpendicular to the longer axis of the leaflet, using a piece of elder-pith as already described for making the section. This section will show us the leaf-tissue or the "mesophyll." First, beneath the upper epidermis, Fig. 61, *ep'*, are the "palisade cells," *pl'*, a double layer of parallel elongated cells containing chlorophyll, perpendicular to the surface of the leaf. We have seen that these were laterally somewhat separated from each other, but we find that the cells of the two layers are joined fast together at their ends. The cells of the second palisade layer, *pl''*, are somewhat less numerous than those of the first, and often two of the outer are united to one of the latter. Next to these layers succeeds a loose tissue of cells which forms a network with open meshes which extends quite to the under epidermis. We call this tissue the "sponge-parenchyma." It has somewhat fewer chlorophyll grains than the palisade tissue. The cells of the upper layer of sponge-parenchyma, *sp'*, are connected with the inner layer of palisade cells and indeed are united to a larger number of palisade cells.

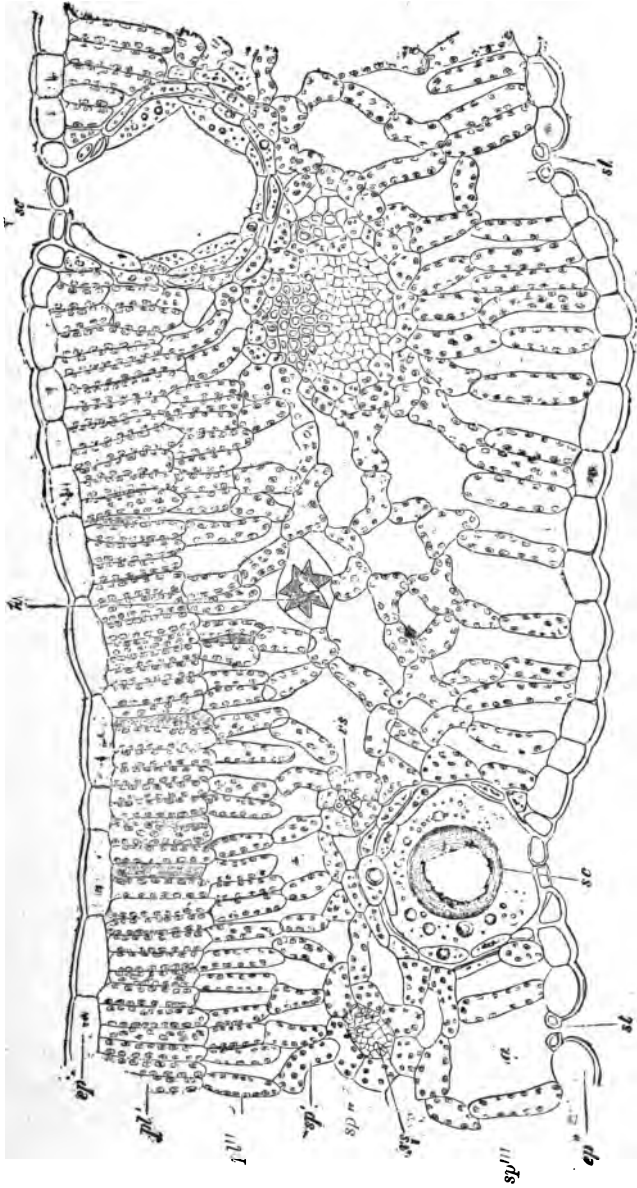


FIG. 61. Transection through the leaf of *Ruta graveolens*. *ep'*, epidermis of the upper side; *ep''*, of the under side; *pl'*, *pl''*, palisade parenchyma; *sp*, sponge-parenchyma; *k*, crystal-bearing cell; *vb*, vascular bundle; *sc*, secretion receptacle; *a*, breathing cavity; *st*, stomata. $\times 240$.

None of the palisade cells are free on their lower ends. When they seem to be, as in some cases in the illustration, it is only that their connecting cells are not in the plane of the image. So also in the sponge tissue there are no cells with free ends, but all are united at their ends. The under layer of sponge-parenchyma, *sp'''*, extends to the epidermis and joins it more or less perpendicularly, thus giving us a form of tissue intermediate between the palisade and the sponge tissue. The breathing spaces, *a*, under the stomata, *st*, are left free. Crystal masses of calcium oxalate, *k*, are found in some of the cells. These cells are swollen, tun-shaped, contain no chlorophyll and seem to be suspended between the green cells. At the edges of the leaflets the epidermis cells are greatly thickened on the outside, the palisade layers are reduced to one and gradually change over into the elongated sponge-parenchyma layer of the under side of the leaf, *sp'''*. The vascular bundles lie in the sponge-parenchyma; the largest, the middle nerve of the leaflet, extends on the one side almost to the inner palisade layer and on the other to the lowest extended sponge-parenchyma layer. In the vascular bundle itself we may easily recognize in the darker part the vessels and in the brighter the bast. The radial arrangement of these elements assures us of the activity of the cambium at some time. About the vascular bundle is a parenchyma sheath whose cells contain chlorophyll grains and to the outer of which the sponge-parenchyma cells are attached. The same relations hold in the smaller vascular bundles represented in the illustration. Still smaller bundles, *vs*, which have but few vessels and bast cells, are met with in the transverse section. They are to the last still surrounded with a sheath of elongated parenchyma cells. The secretion reservoirs, *sc*, touch the upper or under epidermis. They are circular in outline, inclosed by

a layer of thin-walled more or less disorganized cells, upon which borders a layer of flat cells having tolerably strong, white walls and granular contents. Adjacent to these cells is the mesophyll with its chlorophyll contents. The epidermal cells which overlie the reservoir are flat like those which surround it. The volatile oil may be removed by alcohol. A superficial section made at the base of the common petiole shows that the epidermal cells are elongated and on the upper as well as the under side are interrupted with stomata. The oil reservoir is also not wanting. Under the epidermis is a layer of elongated collenchyma cells and next to that the tissue containing chlorophyll. The transection of the petiole shows the epidermis thickened on the outside; beneath this the simple layer of thickened collenchyma cells, which is interrupted only by the stomata. The two or three layers of elongated, green, palisade cells are quite uniformly developed in the whole circumference, but are rather looser on the under side. Within these are, finally, round, first green, then colorless cells which become larger toward the middle. In this inner cylinder, in colorless cells, run the vascular bundles, the largest in the middle nearer the under side, the others in the circumference, diminished in size on both sides and with their wood parts turned towards the middle of the petiole. The larger of these bundles are provided with strings of sclerenchyma fibres. Apparently the activity of the cambium has been more prolonged in these vascular bundles which has produced secondary wood within and secondary thin-walled bast without. The larger vessels appear only in the inner part of the vascular bundles and the border-pitted tracheids in the outer portions.

We will now take a leaf of *Fagus silvatica* for our investigation. On account of the greater thinness of the

leaf it is less easy to get a sufficiently thin section. Take therefore a very small piece of the leaf between the two pieces of elder-pith. Stomata are found only on the under side. Attached to the epidermis, *ep*, Fig. 62, of the upper side, in leaves from sunny localities, is a layer of elongated, palisade cells, *pl*, which are more or less separated from each other by intercellular spaces. They are grouped together in bundles below, and each bundle sets on one or more funnel-shaped, broadened, sponge-parenchyma cells, *sp'*. The latter are connected into a network

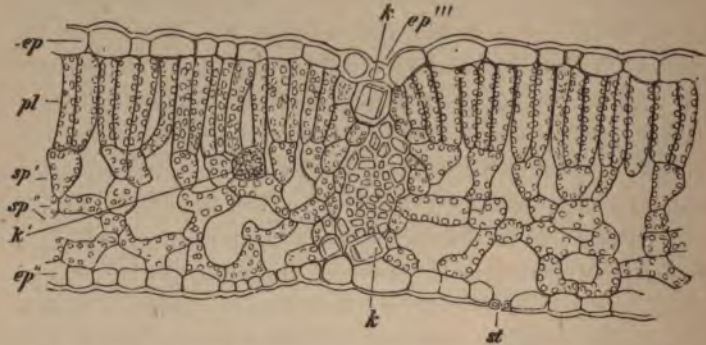


FIG. 62. Transection of a leaf of *Fagus sylvatica*. *ep*, epidermis; *pl*, palisade parenchyma; *sp*, sponge-parenchyma; *k*, crystal bearing cells; in *k'*, a cluster of crystals; *st*, stoma. $\times 360$.

by elongated, sponge-parenchyma cells, which reaches to the epidermis of the underside, *ep''*. Single cells without chlorophyll, but with crystal clusters in them, *k'*, are embedded in the sponge-parenchyma. The principal nerve and the lateral nerves of the first order project from the under side of the leaf as leaf ribs. The projecting portion of the nerve is about as thick as the rest part of the leaf. The vascular bundles extend into the projecting rib. The latter is covered with elongated, epidermal cells

which are followed by elongated, collenchyma cells. To these succeed cells, each of which bears a simple crystal, and then the many-layered sheath of sclerenchyma fibres which encloses the whole vascular bundle. On the upper side, over the vascular bundle, the palisade layer is interrupted in a narrow place and is replaced by collenchyma on which follows a slender stripe of elongated, epidermal cells, *ep'''*. A layer of cells, containing chlorophyll, encloses the sclerenchyma sheath and on them are the spongy-parenchyma.

The ribs represent the mechanical system of the leaf which must be built in conformity to that. The filaments are uniformly apparent in the surface of the leaf, the plane of the filaments being perpendicular to that of the leaf. The upper surface of the leaf is principally stretched by pulling, and the under surface by pressure. The filaments in the present case are I-shaped, the vascular bundles forming the filling of the filaments. The stiffness of the under girding depends greatly upon their springing as far as possible below the under surface of the leaf from the projecting midrib. The nerves expand the blade of the leaf, give it the necessary stiffness and stability and prevent its being torn.

Small vascular bundles, like those in the illustration, are supported only on the upper and under sides with sclerenchyma fibres. The branches of these are without a sclerenchyma layer embedded directly in the parenchyma. The smaller vascular bundles are accompanied both on the bast and wood parts by crystal-bearing cells, *k*. Above and beneath it the epidermal cells are somewhat extended and form shallow, depressed stripes. Long hairs of sclerenchyma fibres grow from the epidermal cells over the nerves, but fall away from the full-grown leaves.

Leaves, growing in sunny situations, are thicker than

those growing in deep shadows (2). The additional thickening comes from an elongation of the palisade parenchyma cells and an increase of the number of the layers. The palisade tissue is thus adapted to greater intensity of light and the sponge-parenchyma to a less. In palisade cells the chlorophyll grains are seen only in profile, on the elongated side walls, protruding less or more according to the intensity of the illumination into the cell cavity. On the contrary, the chlorophyll grains of the sponge-parenchyma are seen in profile or on the surface according to the intensity of the illumination; that is, they take a position parallel or perpendicular to the upper surface of the leaf. The palisade cells first receive the rays of light, while the sponge-parenchyma cells receive it only after it has been weakened by absorption in passing through the palisade cells. This disadvantage is in part compensated for by the sponge-parenchyma cells, exposing the greatest possible amount of surface to it. But if the light become too intense, the chlorophyll grains turn their edges to it. Such leaves which are developed in the bright sunlight are composed almost entirely of palisade cells, while those, only about one-third as thick, grown in deep shadow, consist almost exclusively of sponge-parenchyma.

Still other physiological consideration will be connected with our morphological investigations whose correctness may be tested by the microscopic image.

The assimilation of carbon takes place in definitely colored chromatophores and in the higher plants, exclusively in the chlorophyll grains. Only these plasma bodies have the capacity, in light of sufficient intensity, to disintegrate the atoms of water and carbonic dioxide and form from them combinations which are rich in carbon. This process, taking place mainly in the palisade cells, requires us

to designate them physiologically as the principal assimilation cells. The palisade cells, as we have already seen, are more or less fully, laterally, separated from each other, and below bend together into tufts. So the assimilated matter will not pass laterally from cell to cell, but rather into the widened funnel-shaped cells of the sponge-parenchyma upon which the tufts of palisade cells rest, *sp'*, Figs. 61, 62, the physiological function of which therefore is that of absorbent or collecting cells. From the same point of view the next following cells of the sponge-parenchyma, *sp''*, Figs. 60, 61, may be designated conducting cells. Since the sponge-parenchyma has wide air spaces in connection with the stomata it may be designated ventilation tissue; also as transpiration tissue, since a considerable evaporation takes place from the surface of the cells into the intercellular spaces. It is, on account of its chlorophyll contents, rightly known also as assimilation tissue. The sponge-parenchyma cells are directly attached to the parenchyma sheaths of the vascular bundles and so conduct the assimilated material partly to that and partly to the bast of the vascular bundle. Sheath and bundle together, therefore, are conductors. The vascular bundles also conduct water from the woody part of the plant, giving it out into the surrounding tissue of the leaf, part of which finds its way into that water reservoir, the epidermis. The conducting tissue of the parenchyma sheath about the vascular bundles, much thickened and giving solidity to the "mechanical cells," likewise forms the tissue of the projecting leaf ribs and is known as "nerve parenchyma." This "nerve parenchyma" is continued into the fundamental tissue of the petiole, which, as we have seen in the *Ruta*, is built principally of conducting and mechanical elements. Assimilating cells play but a subordinate part in it.

Let us now study the inner structure of a floral leaf and use the opportunity also to learn of the course and ending of the vascular bundles. Petals of *Verbascum nigrum* are especially well adapted to both of these purposes. The air bubbles which adhere to the petal may be driven out by tapping lightly on the cover-glass. Use no alcohol. We observe a delicate epidermis and from two to four layers of sponge-parenchyma, two at the edges and four in the thicker part of the petal. The stoutest vascular bundles, as well as the finest branches where they are reduced to spiral vessels, are sheathed in a layer of elongated, thin-walled, parenchyma cells. This parenchyma sheath closes together in front over the ends of the vascular bundles. Protoplasmic streaming may be seen in the cells. The stout-branched, sponge-parenchyma cells are joined to the elements of the sheath. Particularly beautiful is the view of the ends of the bundles which exhibit a radiating juncture of the sponge-parenchyma cells on the sheath.

The petals of *Papaver Rhœas* has but one layer of sponge-parenchyma between the upper and under epidermis. The vascular bundles never end free, but rather lock together in commingled arches at the edges of the leaf. They are surrounded in their whole course by a parenchyma sheath of a single layer of cells, to which the sponge-parenchyma cells are joined on both sides.

NOTES.

(1) See Haberlandt, in encykl. d. Naturwiss., Handb. d. Bot., Bd. II, p. 614; J. v. Sachs, Vorlesungen über Pflanzen-Physiologie, p. 59 ff.

(2) See Stahl, zuletzt Jen. Zeitschr. f. Naturw. Bd. XVI, 1883; Concerning the influence of sunny and shady locations on the formation of the foliage leaves.

(3) See Haberlandt, work and vol. quoted above, p. 640.

LESSON XVI.

THE VEGETATIVE CONE OF THE STEM.

OUR next task shall be to select some suitable object which shall make us acquainted with the structure of the vegetative point of the vascular plants. We choose the phanerogam *Hippuris vulgaris* (1) whose vegetative cone is strongly developed and easily prepared. Take a thrifty sprout and cut off a piece from the top about a centimeter long. Remove the larger leaves. Now take the bud between the thumb and forefinger, holding it with the top down and with a razor held perpendicularly, and with a drawing motion, cut the bud longitudinally exactly in halves. Now take one of the halves and in the same way halve it, then the half of this lying nearest the centre of the bud, and so on till a section of sufficient tenuity be obtained. This manipulation may not at first be successful, but it is not a matter of any great difficulty and a little practice ought to make it easy enough.

If, however, one does not overcome the difficulty, he may hold the severed bud between two flat pieces of elder pith and cut as he did between the thumb and finger, but hitting the right point in the object will be much more a matter of chance in this case. But objects of this kind may also be fastened between the edges of two pieces of elder pith and the cut made through them and the pith at the same time, as already explained.

Select a section from the exact middle of the bud, which we recognize by the slender regularly-constructed vegetative cone. This vegetative cone forms the leaves in a many-branched whorl, which may be seen at some dis-

tance from the top as isolated knobs set uniformly about its circumference. Beneath the youngest whorl but one, the node of the stem begins to be indicated by a transverse, thick tissue plate, above and below which, in the rind of the stem, the air passages enter. These air passages, which extend from one node to another, increase in size with the growth of the stem. The internodes grow rapidly and uniformly, both in length and thickness. The vessels of the stem begin to form somewhat below the fourth youngest whorl of leaves. The addition of a little potash lye brings them out very finely. These vessels appear in the longitudinal axis of the stem to belong to the vascular bundle which grows at the extremity and ends at the top in a single ring vessel. The vessels which belong to the leaves make their appearance first in the tenth or twelfth whorl, and are joined to the vessels of the vascular bundle of the stem. At a point not so far removed from the apex, little flat knobs begin to appear in the axils of the leaves which are the beginnings of fan-shaped scales borne on simple, short stipe-cells. Only in plants taken in their blooming season do we find here the axillary buds. In order to study thoroughly the structure of the vegetative cone, we should select a good median longitudinal section, treat it with concentrated potash lye and having washed it, lay it in concentrated acetic acid. After a little while examine it in the same or in potassium acetate. It may be handled to best advantage, since we wish to examine both sides of it, by putting it between two cover glasses and then laying these on a slide, but with no fluid between the lower one and the slide. It can then be turned over very readily. By strong magnification we observe a definite arrangement of the cells in the "meristem" of the vegetative cone. See Fig. 63. There are mantel-like layers of cells whose division walls form a band of

confocal parabolas. The outer layer which runs over the foundations of the leaves and covers the whole cone is the dermatogen, *d*, and forms the epidermis. Under this there are four or more undifferentiated layers of tissue which belong to the periblem, *pr*, out of which the rind of the stem is developed. Finally, we come to a central cylinder with a reduced cone at top, which mostly ends in a single cell, and out of which, as we shall see, by looking deeper into the section, is formed the vascular bundle in the axis of the stem. This tissue we call the plerome, *pl*. Thus the epidermis, rind and vascular bundle of the stem in *Hippuris* have their own "histogen." There is, indeed, no single apical cell but each histogen ends at the top of the vegetative cone in one or more "initial" cells.

It must be added that in all phanerogams, the separation of the histogens in the vegetative cone is by no means so distinctly marked as in this case. In many gymnosperms, *Abietineæ*, *Cycadea*, there is no sharp demarcation between the dermatogen and the periblem and often also the periblem and plerome are not distinctly separated. In the angiosperms the dermatogen is always distinctly set off, but there often exists no boundary between the periblem and plerome. It is not in general a difference of tissue which is continued into the meristem of the cone that gives the necessary stability to the young tissue, but rather the mechanical ar-

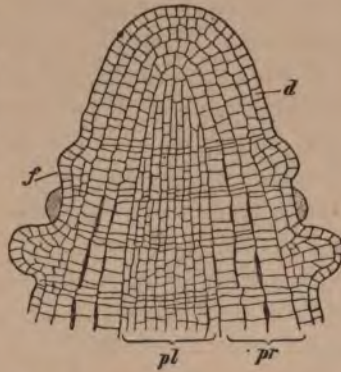


FIG. 63. Longitudinal section of the vegetative cone of *Hippuris vulgaris*. *d*, dermatogen; *pr*, periblem; *pl*, plerome; *f*, beginning of the leaf. $\times 240$.

range of the cell walls. We meet, in this arrangement of the cells, the two sorts of cell division: the anticlinal, that is, perpendicular to the outer surface of the plant, and the periclinal, a division of cells parallel to that surface (2).

We may retain the terms dermatogen, periblem and plerome, in all cases, because the arrangement of cell-layers which we have observed in *Hippuris* frequently recurs in phanerogams, and these terms will serve to designate, therefore, definite regions of the vegetative cone. The epidermis really arises, in the angiosperms, only from the dermatogen. But the vascular bundles may not always find their origin in the plerome, but also in the periblem. In the origination of the leaves we see, as in Fig. 63, first a periclinal parting of the cells and then an anticlinal, in the outer layer of the periblem. The dermatogen remains a single layer even over the arched places, and has only an anticlinal cell-parting. An anticlinal and periclinal division of cells takes place in the periblem layer, in the production of buds, but only an anticlinal in the dermatogen.

For an example of the flat vegetative cone which occurs in most phanerogams, we will select the ornamental shrub cultivated in most gardens, *Evonymus japonicus* (3). This plant may be had at any time of the year and its buds readily lend themselves to section-making. Prepare a transection in order to get a view of the cone from above. First treat the section with potash lye, wash with water and then for a longer time with acetic acid. With a low magnification, we recognize the cone as a flat knob surrounded by the youngest rudiments of leaves. These are arranged in a two-limbed, alternate decussate whorl. Each new pair of leaves comes forth after a corresponding growth of the vegetative cone in the spaces between the two preced-

ing leaves, Fig. 64, *A*. By sufficient magnification the arrangement of the cells at the top of the cone is easily made out, as is seen in Fig. 64, *B*. There is no one end-

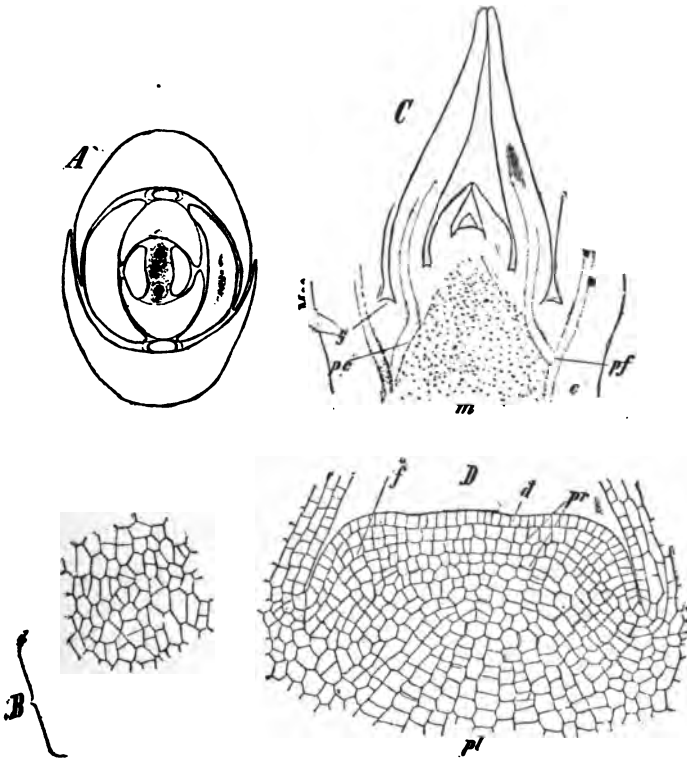


FIG. 64. End of the stem of *Econymus japonicus*. *A*, view from above upon the top. $\times 12$. *B*, view of the apex of the vegetative cone. $\times 240$. *C*, median longitudinal section through the apex of the stem. $\times 28$. *D*, median longitudinal section of the vegetative cone. $\times 240$. *d*, dermatogen; *pr*, periblem; *pl*, plerome; *f*, beginning of the leaf; *g*, beginning of a bud; *pf*, leaf trace; *pc*, procambium ring; *m*, pith; *c*, rind.

cell. A transection, made considerably below the top, shows a rapid differentiation of the tissue, into fundamental tissue, "procambium," which will form the vascular

bundles, and primary rind. The procambium zone appears in cross section as a rhomboid figure, with somewhat projecting and rounded edges. This figure is alternately elongated in the direction of the newly-entering procambium cords. The procambium consists of thin-walled, narrow, radially-arranged cells. The production of the elements of the vascular bundles begins at the edges of the figure, protophloëm elements on the outer, spiral vessels on the inner side of the procambium zone. These regions of the differentiation of the elements of the vascular bundles are not distinctly marked off from the rest of the procambium tissue. The procambium zone opens in places to receive the entering vascular bundles of the leaves. In the axils of the young leaves one may see the beginnings of the axillary buds. The median longitudinal section is shown with slight magnification in Fig. 64, *U*. The flat vegetative cone, the leaf beginnings increasing in size, the axillary buds, *g*, the differentiation of the fundamental tissue, *m*, the procambium zone, *pc*, the vascular bundles common to both stem and leaves, the so-called leaf-trace, *pf*, and the primary rind, *e*, are recognized at a glance. Pith and rind have a large number of crystal masses of calcium oxalate. In a fresh section examined in water, the rind and pith appear green, while the procambium zone is quite clear. Treat with potash lye and acetic acid in order to follow the arrangement of the cells of the vegetative cone.

First, we come to the single layer of dermatogen cells, Fig. 64, *D*, *d*. Next these, mantel-like layers of the periblem, *pr*, and then the plerome, *pl*, a solid central cylinder of tissue not sharply distinguished throughout from the periblem. The vegetative cone appears very narrow between the two youngest embryo leaves, but one may often try many times before he exactly hits the first beginnings of the leaf and makes a section like that represented in Fig.

64, *D*. Then the cone appears much broader and the histogens may be better traced out. The formation of the leaf begins with the periclinal division of the cells in the two outer layers of periblem, *f*, the dermatogen remaining a single-celled layer. The formation of the axillary buds takes place in the same way, in the axils of the third youngest pair of leaves by the periclinal division of the cells of the hypodermal layer. In general, it may be demonstrated that the dermatogen furnishes the epidermis, the periblem the rind, and the plerome the pith of the stem. It is less certain that the procambium ring arises from the plerome.

That the formation of the vascular bundles is not exclusively connected with the plerome follows from the fact that that part of the vascular bundle which enters the leaf is within the rind and is therefore produced by the periblem and that the entire inner tissue of the leaf with its vascular bundles is a product of the periblem.

To illustrate the growth of a cryptogam by an apical cell, we will select *Equisetum arvense* (4). The apical cell is easily seen. Use a growing sprout, taking a fresh one or one preserved in alcohol. Cut off a piece from the top about 10 mm. long, and make a longitudinal section between the fingers as already described. Find a section with the conical tip of the stem intact. Make it transparent by the addition of a little potash lye. Should this be so strong as to make the cell wall too transparent and therefore unrecognizable, weaken the solution by the addition of a little water. In fresh sections we are to avoid the use of all dehydrating substances or we shall shrink up the vegetative cone. Sections from alcohol material may, on the contrary, be examined direct in glycerine, but not after a previous soaking out in water. A section treated with potash may advantageously be stained with

a very dilute solution of safranin. The staining should be very slight and the cell walls will come out all the more distinctly. We get the best results where we treat the section for a short time with concentrated potash solution, then wash with water and lay it for two hours in concentrated acetic acid. Examine in water, or, better still, in dilute acetic acid, or a concentrated solution of potassium acetate. A permanent preparation may be made with the

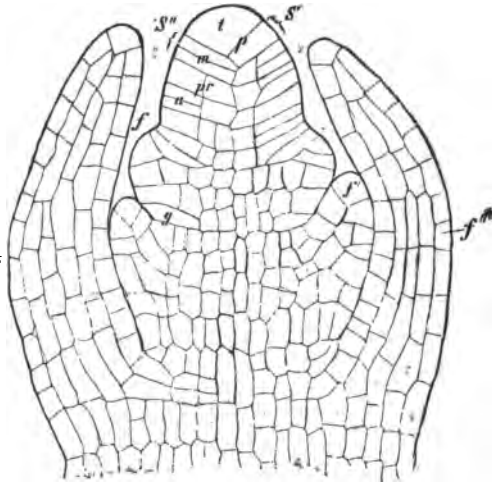
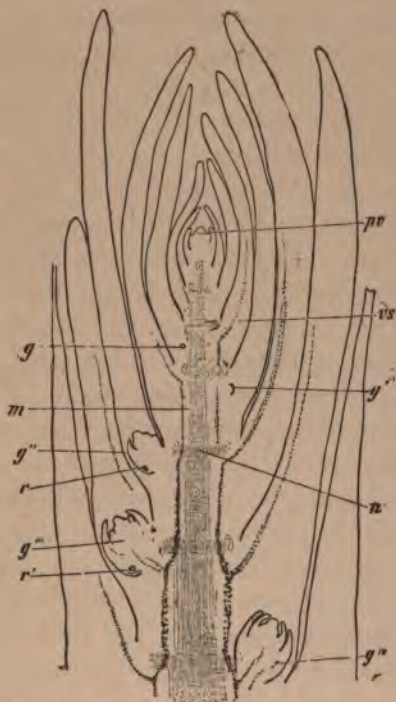


FIG. 65. Longitudinal section of the vegetative cone of a sprout of *Equisetum avense*. *t*, apical cell; *S'*, youngest segment; *S''*, next older segment; *p*, principal wall; *m*, halving wall; *pr*, later periclinal, *a*, anticlinal walls; *f*, first, *f'*, second, *f''*, third leaf whorl; *g*, initial cell of an axillary bud. $\times 240$.

last named fluid. Glycerine shrinks these sections. Examine the section between two cover glasses as already recommended in the case of *Hippuris*.

With a rightly-prepared section the apical cell will appear in the form of a triangular inverted pyramid with a convex base, Fig. 65, *t*. This apical cell divides by walls which are parallel to the existing lateral wall, and which follow each other spirally and form segments arranged in

three exact series. These segments are seen in profile at Fig. 65, *S*. They also divide in a definite way and so the plant is gradually built up. At some distance from the apical cell a wall rises from the vegetative cone which grows at its edge by wedge-shaped initial cells. In its further development the edge protrudes at certain places to form the free top of the leaf-whorl, which grows together at the base. The farther we go from the apical cell, the larger becomes the rudiments of the leaf-whorl, and at the same time the differentiation of the inner tissue of the stem goes on, principally by the separation into thick small-celled low nodes, and thinner long-celled elongated internodes, Fig. 66.



The wide-celled pith next begins to appear. The first ring vessel makes its appearance in the fifth highest internode in the procambium cord on the outer border of the pith and from here may be traced into the beginning of the next higher leaf-whorl. Each single vascular bundle is common to both the stem

FIG. 66. Median longitudinal section through a vegetative sprout of *Equisetum arvense*. *pv*, vegetative cone of the sprout; *g*, initial cell of a bud; *g'*, *g''*, *g'''*, *g'v*, different stages of development of such buds; *r* and *r'*, the beginnings of a root on the buds; *m*, differentiation of the original pith; *vs*, entering spiral vessels; *n*, differentiation of the node diaphragm. $\times 26$.

and the leaf and may hence be called a leaf-trace. As many vascular bundles run downward in each internode as there are leaves in the whorl. The separate leaf-traces first become connected by lateral branches somewhere about the lower half of the seventh internode, thus forming closed vascular bundles. Near the tenth internode the pith begins to become hollow, by the weakening and separation of the cells. In the nodes, on the contrary, the cells of the pith have a corresponding increase and continue coherent. The lateral buds arise from single cells in the axils of the leaf-whorls. They stand in whorls and alternate with the free points of the leaves in the leaf whorls, the tissue of which, at the base, they perforate in growing out. The longitudinal section shows the somewhat larger rudimentary bud growing in the tissue of the thick leaf-whorl which lies upon the surface of the stem. Somewhere near the seventh node the buds are so far developed as to possess several rudimentary leaf-whorls which may be advantageously used for studying the apical cell.

Among the cryptogams only the *Equisetæ* and the *Ophioglossæ* have collateral vascular bundles. The bundles are arranged in a simple ring about the hollow pith. In the wood part of each bundle is an intercellular passage, the carinal cavity. The thin-walled bast portion is enclosed on the sides by the ring and reticulated vessels of the wood part. An endoderm surrounds the whole vascular tissue body. In the broad rind, alternating with the vascular bundles are the wide intercellular passages, the vascular cavities. The number of vascular bundles exactly corresponds with that of the leaf tips in the whorl next above. In order to observe the course of the vascular bundles, make a whole series of transections down through the lower part of the internode, through the node and into the next lower internode. For this purpose we

may use alcohol or fresh material, but should take the youngest possible part of the stem, as the older parts are silicified and soon dull the knife. Use the microtome for this. The sections should be arranged in their order on the slide and be made transparent with potash lye. By an exact comparison of these successive sections we shall learn that each of the vascular bundles, as it descends from the internode above, splits into two branches in the node and each of the branches unites with a neighboring vascular bundle coming down into the node from the leaf-whorl, thus forming a new bundle. If the bundles of the lateral buds are already developed they will complicate the arrangement somewhat. Each lateral bud is connected with the vascular system of the mother axis by two vascular bundles, and indeed with each bundle to the two forking branches of a bundle from the next higher internode of the stem, immediately after it separates into its two branches. The lateral buds alternate with the vascular bundles of the leaf-whorls which cover them, and correspond in their position to the vascular bundles of the next higher and next lower whorls. It follows from our observations that the vascular system is common to the whole plant and is formed of leaf-traces which divide at their base within the node and by means of their forking branches pass into other bundles entering the node from above and below.

As this is the method of the formation of the vascular system generally in vascular plants, we shall limit our studies of the same to this simplest example. In the investigation of a complicated case it is necessary to arrange the successive sections on the slide in the same position for purposes of comparison. This may most easily be done by cutting a longitudinal slit down the side of the specimen which, of course, in each section, will indicate the corresponding side. It is often necessary to draw the

section, in order to be able to demonstrate the shifting of a single bundle with certainty. Tangential, longitudinal sections made transparent with potash may, in many cases, lay bare in a single section, the whole course of a vascular bundle.

NOTES.

- (1) Sanio, Bot. Zeitung, 1864, p. 223, Anm. . . , 1865, p. 184; de Bary, Vergl. Anat., p. 9; L. Kny, Wandtafeln, III Abth., p. 99.
- (2) Sachs, Arbeiten des Bot. Inst. in Würzburg, Bd. II, p. 46, u. 185.
- (3) Hanstein, die Scheitelzellgruppe im Vegetationspunct d. Phanerogamen, p. 9; Warming, Rech. s. l. ramif. d. Phaner.
- (4) See Cramer, Pflanzenphys., Unters. v. Nägeli, Heft 3, p. 21; Reess, Jahrb. f. wiss. Bot., Bd. VI, p. 209; Sachs' Lehrb., IV Aufl., p. 393 und Goebel, Grudzüge, p. 291; de Bary, Vergl. Anat. p. 20.

LESSON XVII.

VEGETATIVE CONE OF THE ROOT.

WE shall now study the vegetative cone of the root (1), beginning with the angiosperms, and taking our specimen from the relatively easy *Graminaceæ*. It furnishes but one of the many possible types of the root growth of the angiosperms, but it is one quite widely distributed and very instructive in respect to the process in question. Take a plant, the common barley, *Hordeum vulgare*, grown in a flower pot. Tilt the pot so as to find the free ends of the roots in the outside of the soil, and make the investigation with fresh material. Make a transection of an old part of the root. We shall find a large vessel in the middle of the axile fibro-vascular bundle cylinder, and arranged about it some eight vascular rays alternating with an equal number of bast parts. The vascular rays extend here to the endoderm, therefore interrupting the pericambium. The endoderm shows more or less distinctly the dark radial shadows. Then follows a pretty stout rind.

Make an exactly median longitudinal section of the end of the root, between thumb and finger as in the other case, and examine without reagents. Before all, make sure that the body of the root is seen sharply distinct from the root-cap. A line may be traced which follows the outer surface of the epidermis over the apex between the body of the root and the root-cap. See Fig. 67. The dermatogen does not, as such, extend over the top, but rather it, *d*, and the periblem, *pr*, come to this point at the top in common initial cells. In the illustration beyond, only one such common cell occurs; there may be several. The der-

matogen may be traced to these initial cells; the periblem, a single layer thick, also touches it. The plerome comes to a point under this cap in its own initial cells. On the

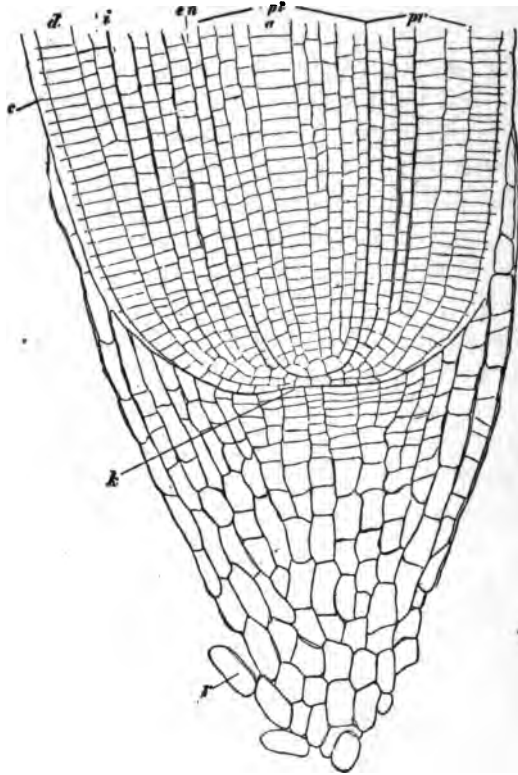


FIG. 87. Median longitudinal section of the end of a root of *Hordeum vulgare*. *k*, calyptrogen; *c*, thickened outer wall of epidermis; *en*, endodermis; *i*, intercellular passage filled with air; *a*, a cell series which will form the central vessel; *r*, disengaged cells of the root cap. $\times 180$.

line which separates the body of the root from the root-cap, toward the outside, are the initial cells of the cap, forming a layer of flat cells and called calyptrogen, *k*.

These cells, in accordance with their origin, are arranged in rows, first flat and then attaining considerable height afterwards, at the top of the cap rounded out and finally separated from each other and become disorganized, *r*. A peculiarity of the *Graminaceæ* is that the dermatogen is strongly thickened on the outside, *c*. It is sparkling white and becomes thicker the longer it lies in the water. On the lateral borders of the cells may be seen strongly refractive strips continued more or less deeply into the thickened outer wall. They are those of the primary walls of the cell and indeed reach further into the thickened walls the deeper they are. These walls show distinct lamination. The periblem has rapidly increased its cell layers by periclinal divisions. Between the inner ones air-filled intercellular passages very soon begin to enter, designated in the illustration by dark lines, Fig. 67, at *i*. The periblem produces the rind, the innermost layer of which becomes the endoderm. The plerome ends in a cone-shaped group of initial cells, two such being discernible in the illustration. The plerome forms the axile fibro-vascular bundle cylinder. The differentiation of the large central vessels in the bundle may be traced quite up to the initial group, the cells which shall form them being indicated by their greater breadth, *a*. Those which shall form the smaller vessels will be first distinguishable much later.

The roots of the gymnosperms show in many connections a peculiar articulation in the meristem of the vegetative cone. Let us study *Thuia occidentalis*. A transection through a full-grown root resembles that of the *Taxus baccata* only that the root of the *Thuia* is quadrilaterally built. A median longitudinal section through the end of the root shows a well-defined plerome cylinder which ends in a few initial cells and is surrounded by a mantle of per-

iblem twelve to fourteen layers of cells thick. The latter continues over the end of the root and forms there its inner series of eight to ten colored initial layers while the outer series passes over into irregularly-arranged relatively-large cells. These large cells extend to the top of the root-cap where they finally separate and fall away. The root-cap of *Thuia*, as of the gymnosperms generally, consists of the outer elements of the periblem, dermatogen and calyptrogen both falling. The initial layers of the periblem divide by both periclinal and anticlinal walls. The periclinal division increases the number of periblem layers and supply from within the cells thrown off from the periphery. The anticlinal divisions increase the number of cells in the single layers and provide principally for the building up of the rind. The periclinal dividing, in the initial layer of the apex, produces this result: that the cell series of the rind when followed out to the point seems to be doubled. The central straight anticlinal cell row in the periblem of the apex of the root is much more distinctly conspicuous than the neighboring cells. It forms the "periblem column" which loses itself in the brown cells of the root-cap. This column appears clearer, its cells immediately touching each other, while they laterally form adjacent air-filled, intercellular spaces. These cells are also distinguished by their rich starch contents. The roots of the *Thuia* possess no epidermis, the surface of the root being covered with the outer layer of the periblem. If we follow this layer in the direction of the end of the root, we shall soon find it extending under another which now, for some distance, constitutes the outer surface. This outermost living cell layer is protected on its outer surface by the collapsed and browned cell walls of a dead cell layer. The roots of *Thuia* and of the gymnosperms gen-

erally have no root hairs. Fig. 68 shows a slightly magnified image of a longitudinal section through the end of the root in which the various parts can be easily made out. We see first the brown cell-sheath, *x*, then the periblem, *pr*, which extends over the apex of the root and whose outer layer there forms the root-cap; finally the plerome, *pl*, whose upper termination is not clearly seen with so small a magnification. One may easily think the upper part of the plerome to be more bulky than it really is, because the innermost layer of the periblem borders on the plerome without an intercellular space and appears as clear as the plerome cylinder itself. In the oldest part of the section the plerome cylinder is covered by a layer of red cells, which correspond, as a comparison with the transection shows, with the endoderm cells filled with red cell-sap. At some distance from the end of the root these cells are still unrecognizable. Vessels, *s*, are formed in the older parts of the plerome cylinder. The apex of the periblem is occupied with the conspicuous periblem column, *c*, against which, laterally, the layers of periblem abut. The latter extend neither to the plerome nor to the outer surface of the root, which last is covered by large brown cells.



FIG. 68. Longitudinal section of the end of a root of *Thuia occidentalis*. *x*, outer brown layer of cast-off cells; *pr*, pericambium; *pl*, plerome; *e*, endoderm; *s*, spiral vessels; *c*, periblem column; *k*, root-cap. $\times 26$.

We will make use of a coniferous plant in studying the methods of root-branching. We observe in the roots of

Thuia occidentalis that they bear lateral rootlets in four, and at last in three, straight rows. By making a section of the root we find that these rows of rootlets correspond first to the four- and then to three-sided vascular bundle cylinders in the roots. By making a section through the

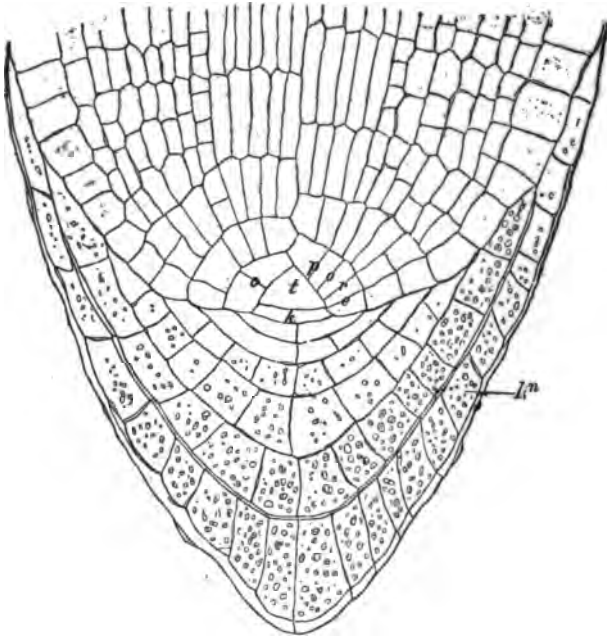


FIG. 69. Median longitudinal section of a root of *Pteris critica*. *t*, apical cell; *k*, initial cell of cap; *k^o*, outer cap; *c*, *e*, *r*, *p*, cambium, epidermis, rind and pericambium walls respectively. $\times 240$.

root at the point of insertion of the rootlet we find that the rootlets stand before a wood part of the cylinder, and since these wood parts run straight along the axis of the vascular cylinder the observed arrangement of the lateral rootlets is explained. We will now undertake to learn

something of the vegetative cone of a root which grows by means of an apical cell (3) There is no such variety of forms in the roots as in the stems which grow by this means. The three-sided pyramidal apical cell occurs, however, and the articulation by the formation of segments remains constant. Take the root of *Pteris critica*, Fig. 69. It would be quite as well to select another form. By tilting the flower pot we shall easily obtain, uninjured, the end of the root. The roots of *Pteris critica*, as of ferns generally, are bilaterally constructed, flat bast parts alternating with the wood; the pericambium consists of one layer, the endoderm is flat and the inner part much thickened. Prepare a median longitudinal section as already directed. It is not difficult to bring the apical cell here into view. It does not take in the apex of the root, but is covered with the root-cap. The apical cell, Fig. 69, *t*, like that of the stem of *Equisetum*, has the form of a triangular pyramid whose convex base is turned toward the root-cap, while the apex is sunk in the body of the root. The divisions succeed each other as in the *Equisetum* parallel to the lateral surfaces, but besides this there will occur from time to time, mostly after every three of the described divisions, the formation of a wall in the direction of the surface of the base. The cell produced by this has nearly the form of a segment of a globe. This cell, *k*, is an initial cell for the root-cap, forms a cap-like cell layer and is the origin of the root-cap. It divides into halves by a wall perpendicular to the under surface. These halves repeat the division, thus forming four four-sided cells. By a constant repetition of this process of division, that is by walls perpendicular to the basal wall, an old cap, *k*^a, will consist of a large number of cells. The cells of old caps are filled with starch grains. They become grad-

nally disorganized, while the apical cell constantly produces new initial cells. The outer walls of the, for the time being, outer layer of cap-cells become much thickened. The division walls formed parallel to the sides of the apical cell follow, as in the stem of the *Equisetum*, the direction of a spiral.

NOTES.

(1) Sachs, Lehrb., iv Aufag., p. 166; v. Janczewski, Ann. d. sc. nat. Bot., v Sér., T. xx, 1873, p. 162ff.; Treub, Musée bot. de Leide, T. II, 1876; de Bary, vergl. Anat., 1877, p. 10.

(2) Strasburger, Coniferen und Gnetaceen, p. 340; de Bary, vergl. Anat., p. 14. See there also the further literature.

(3) Nägeli u. Leltgeb, in Beitr. zur wiss. Bot., 4 Heft, 1868, p. 74ff.

LESSON XVIII.

HISTOLOGY OF THE MOSSES.

HERETOFORE we have studied the structure of the stem and leaves in the vascular plants only. We will now turn to the small stems and leaves of the mosses, which are quite without vessels (1). We will begin with *Mnium undulatum*, a relatively complicated case, in which the differentiation of tissue is quite well advanced. Make first a delicate transection through the stem. In the middle of the stem is an axillary cylinder formed of narrow thin-walled cells. We may consider this cylinder as the simplest "conducting bundle." Its cells have no living contents, but contain water only. They are distinguished from the surrounding cells by the yellowish-brown color of their walls. Upon the conducting bundle about the wide cells of the rind, which are much larger, with greenish-yellow walls, and living, chlorophyll-containing contents. They increase somewhat in width towards the outside but at the periphery become suddenly narrow and thick-walled, and pass over without definite demarcation into the epidermis, which consists of one or two layers of much thickened cells. In two or three places the outer layer of cells of the stem is continued into a cell-plate of a single layer, which corresponds to the downward running leaf-wing on the stem. A section made below the leaves in the stouter brown part of the stem shows the walls of the peripheral cell layer colored a dark brown. From single cells of the surface grow long, brown-walled, many-times-branched cell fibres, designated root-hairs or rhizoids, which do duty as roots. These rhizoids are distinguished by oblique division walls,

and are hence an exception to the general rule which would demand an exactly transverse wall. Under many such division walls and indeed beneath their elevated edge spring wider spreading lateral branches. Only the growing ends of the rhizoids have colorless walls.

These root fibres exhibit the greatest resemblance in respect to branching, and the inclined division walls, to the primary growth, the so-called protonema, of the typical mass, which is first developed from a sprouting spore. Still these branches, when they do not penetrate the soil, are colorless and bear chlorophyll grains. The leaf buds which develop into moss stems are lateral branches of this protonema. The near relation of rhizoids and protonema is seen also from the circumstance that the rhizoids dampened and set out in the light can produce protonema which will give rise to numerous new plants. It is only necessary to lay a tuft of *Mnium* bottom side up and keep it damp in order to produce a rich green protonema mass from the rhizoids, which resembles terrestrial *Vaucheria* tufts in its general appearance.

If the section should be made through some point in the stem of the *Mnium* which had been injured we shall not find the injury repaired by being closed up with a layer of cork, for the cryptogams with the exception of *Botrychium* cannot form cork, but the walls of the adjacent cells will be thickened and browned so that they will, with the exception of their greater interior diameter, resemble the other cells of the outer surface.

The transection will show near the surface of the stem single small strings of thin-walled cells which agree in color and in their function as carriers of water, with the cells of the central cylinder. These are the conducting bundles belonging to the leaves and end blindly in the rind of the stem. In *Polytrichum*, however, they extend

further inward and connect themselves with the central conducting bundle of the stem. Put a leaf in a drop of water on the slide. We shall find it to consist of a single layer of cells with a middle nerve of several layers, the latter ending in a terminal tooth which consists of a number of rhombic cells. The cells of the mid-rib are much elongated, and the outer ones contain chlorophyll grains. The cells of the leaf are polygonal and also contain chlorophyll. The bandlike hem around the edge of the leaf is formed of elongated, much-thickened cells. At nearly regular intervals on the outer edge are sharply pointed teeth one or two cells long. We may get sections of the leaf at the same time that we make sections of the stem. But if we wish to make sections of the leaf separately, we may fasten a number of them together with glycerine gum, and without waiting for the gum to dry, make sections of the whole between pieces of elder pith. Then lay the sections in water and the gum will be dissolved. This method is recommended for very thin flat sections. We see from one section that the leaf consists of one layer of cells and that the cells of the leaf-hem are very much thickened. The nerve projects more on the back than on the front of the leaf, and in the middle of it somewhat nearer the under side lies a string of thin walled cells, the conducting bundle which we before saw in the rind of the stem. This string is protected behind by some much thickened narrow cells. The image reminds us not a little of certain much reduced monocotyledonous vascular bundles, which consist of a few bast elements and a thin layer of sclerenchyma cells.

If the stem of a wilted plant be put in the water the plant will remain wilted, but if the leaves be put in the water the plant will rapidly become turgescient. The leaves, therefore, are the principal absorbents of water, which fact

renders a direct connection of the conducting bundle of the leaf with that of the stem quite superfluous.

The turf moss offers certain striking peculiarities which we will now consider. Make a transection of the stem of *Sphagnum acutifolium*. The section shows us a wide central cylinder consisting of wide somewhat collenchymatously thickened cells; towards the periphery the cells become gradually narrower, and in the outermost layer are colored a yellow brown. There is no specialized conducting bundle in the interior of the cylinder, which is inclosed by an outer rind of large cells three layers thick. These cells lie next the narrow yellow-brown cells of the inner cylinder. They are distinguished by their large round or oval orifices and their delicate spiral bands. The openings in the walls really connect the cell cavities of adjacent cells as may be seen when the section touches one of them. One often sees the mycelium of a fungus passing through these openings from cell to cell without hindrance. These porous cells of the outer walls of *Sphagnum* contain only water or air and have no living contents. They serve the plant only as capillary apparatus by which the water is conveyed to the place where it is to be used. The plant has no cutinized cell walls; concentrated sulphuric acid dissolves the whole tissue, but the middle lamella and the pores of the yellow-brown outer cells of the central cylinder resist the action of the acid longest.

The extended leaf is ovate, bordered, one layer of cells thick, and consists, as a superficial view will teach, of two kinds of elements: one, of living cells with protoplasm nucleus and chlorophyll grains; the other of dead cells filled with water or air, and furnished with rings or spiral bands and openings between the cell cavities. The reason why dead cells used to carry water or air so often have their cell walls strengthened with spiral bands, rings

or reticulations is because they have lost their turgidity and must have some such mechanical support for their walls in order not to collapse or become compressed. The green cells of the leaf-blade are all connected together and form a network with elegant, bent walls whose meshes are occupied each by an empty cell. The green cells serve for the assimilation of carbon, the empty ones, like those of the stem, for conducting water. The outer edge of the leaf is occupied by slender green cells, and at the conclusion of these, by a slender border of cells, a single layer thick, bearing watery contents, slightly thickened on the outside and somewhat collapsed. Only the ends of these cells seem much thicker and project a little.

There is no nerve in the leaves as there is no conducting bundle in the stem. The plant is therefore much more simply constructed than the *Mnium* in this respect, but more complicated, on the other hand, in being provided with a special capillary apparatus.

The well-known *Marchantia polymorpha* (2) presents a pretty complicated structure. The lack of a cormophytic articulation does not necessarily imply a simple anatomical structure. The thallus is hard and leathery. It branches by the forking of the growing point which lies at the bottom of the apical sinus. If a sprout has but recently forked, the middle of the anterior indentation will be occupied by a thallus-lobe, at the two sides of which lie the apical sinuses. In the middle of each lobe on the under side, an indistinctly-outlined mid-rib projects. Stripes run out diagonally forward from these, bending toward the edge of the frond. At some distance from the end, fine rhizoids spring from the middle of the thallus and serve to fix it to the ground. By examining the under side of the plant, under the simplex, we can demonstrate, by the help of a needle, the presence of scales springing

from the surface of the thallus. There are three distinct forms of these ventral scales: those which grow on the edge of the frond, those which grow in the middle and those which are inserted between. The second and third sorts of scales give the stripes which we have observed with the naked eye. Viewed with a lens, the back side of the frond appears to be divided into small rhomboid fields, the boundaries of which are dark green, the fields themselves more grayish. In the middle of each is a minute opening. Examining a section made parallel to the back

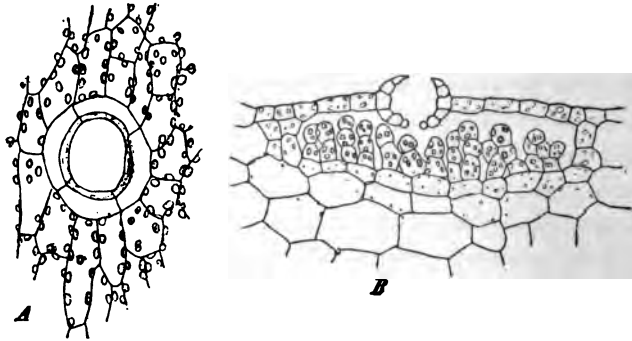


FIG. 70. *Marchantia polymorpha*. A, stoma seen from above; B, in transection.

side of the thallus with a higher magnification and we see that the outer cells are polygonal, closely connected and contain numerous large chlorophyll grains. The round opening in the middle of each field is bordered by, at most, four slender, bent, sickle-form, chlorophyll-free cells, Fig. 70, A. When the section reaches deeper, air collects under the surface of the fields. Into these air cavities, "air chambers," chlorophyll-containing cell fibres project. The lateral walls of the air chambers are constructed of closely connected cells, one to several layers thick and contain chlorophyll. In single cells of the surface are

seen certain bodies characterized by their strong refractive power, their irregular outline and cluster-like form. In young shoots these bodies are faintly brown, in older, brown, containing mostly only fatty oil and form the so-called "oil bodies" of the liverworts (3). A superficial section made from the under side of the thallus shows no fields, while the cells are elongated and contain less chlorophyll than those of the upper side. The rhizoids exhibit a double structure. They are slender and provided with conical projections, or thicker and without these.

The former take their rise from that portion of the frond covered by the middle and the intermediate scales or by the former only. They lie close to the frond, quite up to the middle nerve, are covered with the scales and serve to stiffen the thallus. The common rhizoids arise principally from the middle nerve and turn with a uniformly acute angle toward the substratum upon which they fasten the thallus. At their points they are often lobed and at their base colored purple. All ventral scales consist of one layer, the middle of living, the other two of dead cells.

A cross-section of the thallus shows it to consist on the upper side first of a zone of chlorophyll-containing tissue, then within, of wide cells almost destitute of chlorophyll. On the under side, the last two layers of cells are again narrow, flat, and rich in chlorophyll, forming the so-called "ventral rind layer." Oil bodies are scattered through the whole tissue. Muciperous cells, which are distinguished by their size and refractive qualities, are but poorly developed in the *Marchantia*, but much more richly in other related genera. Looking now at the upper portion of the transection, we first see a simple layer of flat cells which over the air chamber are set free on the walls which form the sides of the chamber. In the middle of the free outer wall is the breathing place, which, as it now shows

itself, is inclosed by several, from four to eight stories of cells (4). See Fig. 70, *B*. The opening is narrowed above and below. The cells of the upper layer are elongated into a membranous border. Get the air all out of the breathing places, if possible, since it very materially injures the image. Branched cell fibres, two or three cells high project into the air-chamber, arising from the next lower layer of cells which are flat and mostly free from chlorophyll. On the lower side of the thallus at the middle nerve, are the lateral, alternating, overlapping scales. Between the scales lie the transections of the bundles of rhizoids. A middle longitudinal section shows the insertion of the stronger common rhizoids which uniformly descend from the thallus, and the cone-bearing rhizoids.

Metzgeria furcata (5) is a very simply constructed thallus and in many respects very instructive. The inconspicuous plant is widely distributed, and on the bark of deciduous trees is not difficult to find. The thallus is ribbon-shaped, clear green dichotomously divided, and has a mid-rib distinguishable by the naked eye. Aside from this mid-rib the thallus consists of one layer of cells, which are polyhedral, and filled with long chlorophyll grains. The slender mid-rib projects much more on the under than on the upper side. It consists, as one may see, by focussing down through it, first of broad and but little elongated cells, then of slender elongated, and finally again of broad cells. The two outer layers contain chlorophyll. At the vegetative point on the under side of the nerve are a few short club-shaped hairs, filled with a strongly refractive contents. Out of the older parts of the nerve and also out of the marginal cells of the thallus come the so-called bristle hairs which, under favorable circumstances, form a lobed holding-disk and thus serve as rhizoids. They are always placed on the posterior end of the cell from

which they are separated by a bent division wall which does not pass through the whole height of the cell, but rather cuts off but a corner or edge of it. The inner cells of the mid-rib are somewhat strongly thickened, with almost collenchyma sparkling-white walls. The dividing process at the vegetative point may be followed in *Metzgeria* in the easiest and most instructive manner (6). In

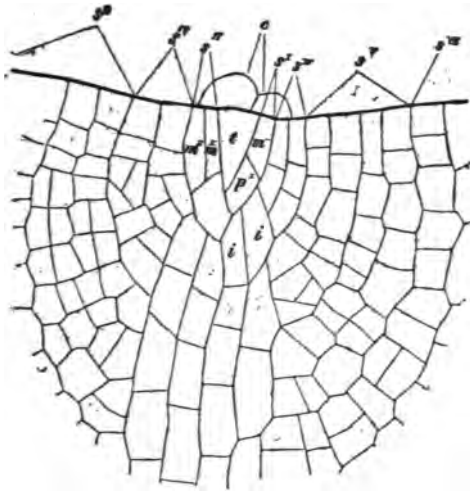


FIG. 71. Terminal growth of *Metzgeria furcata*. *t*, apical cell; *s*¹—*s*ⁿ, series of consecutive segments; *m*¹ and *m*², marginal cells of the first and second order; *p*¹, flat or outer midrib cells of the first order; *i*¹, inner cells of the midrib; *c*, club-shaped hairs. The picture is made with the lens focussed on the inner cells of the midrib. $\times 540$.

the *Metzgeria* the growing point is but very slightly re-entrant. The bottom of this apical sinus, exactly at the point where the mid-rib ends, will be occupied with the apical cell. Examine it from above so as not to be disturbed by the club-shaped hairs. The apical cell is two edged, Fig. 71, *t*, and has the form of an isosceles triangle,

with the base towards the front, mostly somewhat convex and the sides slightly bent. It is divided by walls which run parallel to the lateral walls and gives off segments alternately right and left, which consequently all lie in one plane.

NOTES.

(1) See P. G. Lorentz, *Jahrb. f. wiss. Bot.*, Bd. vi, 1867-8, p. 363; Goebel, *Grundriss der systematischen und speciellen Pflanzenmorphologie*, 1882, p. 184; there also the literature p. 179. Later studies also G. Fritsche, *Ber. d. deutsch. bot. Gesell.*, 1 Jahrg., p. 83 und Haberlant; the same, p. 263.

(2) See Leitgeb, *Untersuchung über die Lebermoose*, vi Heft, 1881. There the rest of the literature.

(3) Pfeffer, *die Oelkörper der Lebermoose*, *Flora* 1874, No. 2.

(4) Voigt, *Beitrag zur vergl. Anat. der Marchantien*, *Bot. Zeitg.* 1879, sp. 729.

(5) See Leitgeb, quoted above, Heft III, p. 34. There also the other literature.

(6) See Kny, *Jahrb. f. wiss. Bot.* Bd. IV, p. 85.

LESSON XIX.

HISTOLOGY OF THE FUNGI, LICHENS AND ALGÆ.

STAINING THE CELL CONTENTS.

THE vegetative organs of the *Fungi*, with the exception of a few of the simplest forms, consist of threadlike, elongated, more or less elaborately branched cells, the "hyphæ" so called. They are either with or without division walls. The most massive fungus bodies consist of aggregations of this hyphæ. The hyphæ may really be so solidly united into a mass as to form a tissue, called pseudoparenchyma which very strikingly imitates the appearance of the parenchymatous tissue of the higher plants. Still this pseudoparenchyma is the product of a union of cell fibres and not of the progressive division of cells in three directions. For the study of this kind of structure we will take the fruit body of a toadstool, *Agaricus campestris* (1), a plant found the year round and of comparatively simple structure. First make a longitudinal section of the pedicel of a full grown plant. We find a structure of longitudinally running hyphæ, which we can easily unravel in that direction with a needle. The hyphæ are arranged more or less parallel with each other, single ones occasionally running obliquely between the others. Each hypha forms a cell thread which is laterally branched, here and there by branches which spring from under the division walls or else farther down along the side. Sometimes the cells of neighboring hyphæ are connected by a cross branch, and communicate openly with each other. At the periphery of the stem the hyphæ are slenderer, more closely compacted together and on the surface their walls are brown, and their inner cavity more or less perfectly collapsed.

Towards the middle of the pedicel the hyphæ become likewise slenderer but their texture is more loose and their course altogether irregular. Large quantities of air fill the spaces between the hyphæ. Until the disturbing influence of the water on the cell contents has made itself felt, little is to be remarked of it; sometimes on the transverse walls a collection of it may be seen. Afterwards large vacuoles form in the cells. Infrequently small crystals may be found in the cells.

A transection of the pedicel gives a parenchymatous appearance which is lost only in the middle



FIG. 72. *Agaricus campestris*. Part of a transection of the pedicel. Two of the hyphæ are cut near the division wall, a dot appearing in them. \times 540.

of the section where the hyphæ present their sides to view. This pseudoparenchymatous tissue appears to be formed from cells of various sizes irregularly polygonal, with more or less numerous intercellular spaces and openings between them, Fig. 72. Sometimes the section will cut close to the transverse wall in which case a point will be seen in the middle of the cell. See Fig. 72. It is a pit which is covered on each side of the cell wall with a small collection of light-refracting substances. Such pits in the centre of the cell wall are quite common in the *Basidiomyceteæ* and the *Ascomyceteæ* (2). In the protoplasmic wall-lining of the cells are numerous very small nuclei but which, not being easily seen, we will not further consider.

For a knowledge of the structure of the frond of the lichens we will select *Physcia ciliaris* found on tree-trunks everywhere. The thallus is an ascendant leafy bush, on the back gray green to living green, on the front gray. Stiff hairs grow from the edges, are often forked and when they reach the substratum grow to it. Make a

section between pieces of elder pith and examine with a sufficiently high power, and we shall see that the thallus consists, on the back side, of a compact layer of narrow, thick-walled hyphæ, the rind layer. Farther inward the hyphæ wind about each other in order to make the loose tissue of the fundamental layer. Here it is easy to demonstrate that the hyphæ are long, branched tubes jointed by division walls. On the border, between the rind and pith, are comparatively large green round cells, the gonidia. They are the same as the algæ *Cystococcus humicola* Nägl.

The hyphæ lie about the gonidia and carry to them raw nutritive substances, of which they receive back a portion when it has been assimilated by the gonidia. There is here, therefore, a case of communal life between the fungus and the alga by which they are mutually serviceable to each other. On the underside of the thallus the hyphæ are in this species again closely interlaced to form a lower rind, or the loose fundamental tissue extends to the lower surface, the latter being the most prevalent case; but, on the edges, the rind-layer of the back of the frond passes around to the front side, in all cases. From these edges the hold-fasts or rhizines grow, consisting of parallel hyphæ closely fastened together. The walls of these hyphæ are brown. This string of fibres is often divided at the base. In other lichens, these rhizines grow from the lower surface of the thallus. Chloriodide of zinc solution immediately colors the gonidia a beautiful blue, while the hyphæ take only a yellow or a yellow-brownish color, the so-called fungi-cellulose reaction.

The thallus of the plant before us is said to be heterogeneous because the gonidia are distributed in a distinct layer. The more highly organized lichens have a homogeneous thallus, the gonidia being evenly dispersed through

the whole frond. Among the latter are the gelatinous lichens, in which the gonidia are embedded in a gelatinous mass through which the hyphæ of the fungus freely penetrate. The algæ which participate in the formation of the lichens are of different species, are green or blue green, but belong exclusively to the lowest groups of these plants.

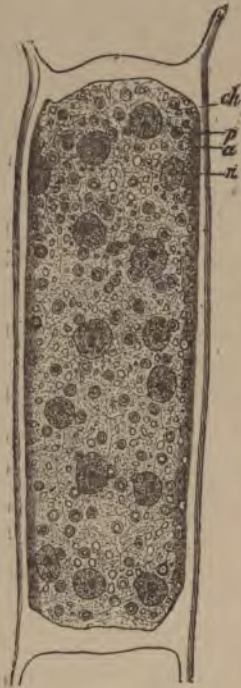


FIG. 73. *Cladophora glomerata*. A cell from a filament prepared with chromic acid and carmine. *n*, nucleus; *ch*, chromatophores; *p*, amyloplum-centres; *a*, starch-grains. $\times 540$.

The *Cladophora* (3) furnish us a much branched green thread whose thickness diminishes with the degree of its branching. It is a fresh-water plant widely distributed and every species is adapted to the investigation. The determination of species in this genus is very difficult. We will select the dark green, floating, tuft-forming *Cladophora glomerata*, for particular study. It is branched in a bushy form, the lateral branches springing from the upper end of the cell. The branching is acropetal so that the end cell of the branch may be considered as the apical cell. But there arise also from the older joints additional branches, in a certain sense adventive branches.

By sufficiently powerful magnification the wall lining is seen to be formed of small plates, Fig. 73, *ch*, which are laterally separated by delicate colorless lines. In each plate are several colorless granules, *a*, besides which in several of the plates are relatively

larger, globular, strongly refractive forms, in which are to be distinguished an inner nucleus and an outer envelope, formerly called amyllum centres, but more recently "pyrenoids," *p.* (4) The cells are filled with cell-sap and divided into irregular polygonal chambers of various sizes, by colorless extremely thin plasma plates which extend from the wall layer through the cell cavity. These plates sometimes contain chromatophores. By careful focussing, colorless plasma balls may be seen on the wall-layer projecting into the cell-cavity. They are nuclei in which, with a specially favorable position, nucleoli may be made out. In this plant we have a multinuclear cell. If now we press upon the preparation pretty smartly, we shall see the wall layer forced back a little, and the chlorophyll plates separated from each other and rounded out. At the same time the small grains and amyllum centres stand out distinctly in the chromatophores, which now seem to be affected by the water the same as the chlorophyll grains of the higher plants. If we now add a solution of potassium iodide of iodine the small grains and also the outer covering of the amyllum centres will be tinged violet, but in the green chromatophores, and also the occasionally visible nuclei, a brown color appears. We must not fail to seek in this preparation for uninjured cells, in which starch grains and amyllum centres are stained and stand out sharply in their natural position and in which also by deeper focussing we distinctly make out the nucleus. Sufficiently strong magnification gives us the angular forms of the albumen crystals (5), of which two will sometimes be found in an amyllum centre. In a short time, in the chlorophyll plates, are seen irregularly formed brown grains, which come from the disintegrated chlorophyll coloring matter, and present us the hypochlorine, or chlorophyll reaction (6). The reaction may be had from the influence

of other acid salts,—but we must adopt other processes for studying the nucleus more exactly, and getting a look at its method of parting. This will give us the best opportunity to get acquainted with some approved methods of fixing and staining, to which histological studies owe so much in recent times. Put parts of the plant in 1% solution of chromic acid, in concentrated picric acid, in 1% solution of chromic and acetic acid, 0.7% of the former and 0.3% of the latter, respectively (7). Let the first and last stand several hours. No harm will come in twenty-four hours. The second may stand twenty-four hours. Then wash carefully in distilled water. They may be kept in water for a whole day changing frequently. The picric acid preparation requires very careful handling if it is to be stained with hamateïn ammonia. The variously fixed and well washed preparation we now lay in Beale's carmine in watch-glasses (8), in Thiersch's or Grenacher's borax-carmine, and in Hoyer's neutral carmine. The plant should be subjected to the action of Beale's carmine twenty-four hours, half that time to Hoyer's, several hours in the borax-carmine. Another part of the plant we will stain with Grenacher's or Bohmer's hæmatoxylin which to stain well should be as old as possible and should be used very dilute. It is best to test the staining from time to time by examining with the microscope, and when the requisite degree of intensity is reached to take it out of the solution. If, in spite of this precaution, the color becomes too dark, it may be put in pure water, or in a solution of alum, or in water containing a trace of muriatic acid, till the required shade of color is obtained. If acid is used in removing the color, the specimen should be afterwards transferred to a weak solution of ammonia for a few minutes. In order to stain the preparation with the ammoniacal hamateïn method (9) we must remove the last

trace of the picric acid by putting it in a large quantity of well boiled water, which we repeatedly change, for from twenty-four to forty-eight hours. For the preparation of the staining fluid we throw some hæmatoxylin crystals into a small quantity of distilled water and blow upon it a jet of ammonium gas. This is done by means of a wash bottle containing an ammonia solution, in which the two glass tubes do not reach the fluid. The crystals dissolve with a beautiful violet color. Dilute the solution with distilled water and let it stand two hours. The right shade of color may be determined directly, but it is well perhaps to make the color too high and weaken it by immersion in water for several hours. This method of staining requires care but it gives the most satisfactory results. Preparations hardened with anything else than picric acid are less suited to this staining. The other named carmine stains are most beautiful if they are over colored and then laid for some time in a watch glass with 50 to 70% alcohol, to which a drop of hydrochloric acid is added. For this purpose one may keep on hand a $\frac{1}{2}$ % solution of hydrochloric acid in 70% alcohol. If the preparation has a more or less diffused stain, the addition of the acidulated alcohol will give it a sharp stain. The preparation should always be washed in alcohol after treatment with the acid-alcohol.

If we wish to make permanent preparations of our stained objects we will select for the carmine preparations glycerine or glycerine-jelly or Hoyer's mounting fluid. If we use the glycerine or the glycerine-jelly for hæmatoxylin stains we must be sure it contains no trace of acid. The Hoyer mounting fluid is also well suited to hæmatoxylin stains. The preparation should not be put directly into the mounting fluid, else the cells will collapse by the too sudden withdrawing of the water, but it should first be put in very dilute glycerine which concentrates slowly by stand-

ing in the air where the water may evaporate. Then the plant may be transferred to glycerine or glycerine-jelly or Hoyer's mounting fluid without damage. The glycerine preparation should be cemented with Canada balsam. The other media named will need no cementing.

Considering now the different fixing and staining media for preparations we may in general say, that chromic acid or its mixture goes best with the carmine stain; and picric acid for fixing with the hæmatoxylin or the hamateïn ammonia staining fluid. But it must be expressly emphasized that these results are restricted to the present object, and that other objects may be better treated by other methods. It also frequently happens that an already tested staining fluid, for some unknown reason fails, so it will not be safe to base a conclusion on a single case. Generally the fixing and staining of cell contents is an art which can be learned only by practice, and one's first attempts are often failures. We have chosen the *Cladophora* as one of the most suitable objects for the introduction of the different hardening and staining processes. He who will follow the method given here, strictly, will seldom fail; hardening with 1% chromic acid, and staining part with borax-carmine, and another with hæmatoxylin.

In the borax-carmine preparation, Fig. 73, the nucleus comes out very sharply. The amyllum centres and the rest of the cell plasma remain as good as uncolored and the starch grains take no color. Within the amyllum centres, the albuminous crystals are quite distinct surrounded by a hollow globe which, as we saw, gave the starch reaction with iodine. The nuclei are distributed quite uniformly through the cells lying in the chlorophyll layer and projecting into the cell-mass. The nucleus shows a darkly stained nucleous and, for the rest, seem to be finely granular or minutely porous. The hæmatoxylin or hamateïn prepara-

tions have the nucleus colored dark, also the crystals in the amyllum centres, even if but slightly. The starch grains are not colored, but the microsomes of the cell-plasma are, and almost as the crystals.

The genus *Spirogyra* presents us with a simple filamentous cell. We will choose a species which has a central, easily visible nucleus, *Spirogyra majuscula*, which is sporadic but not rare in ponds. Other species with central nucleus will serve and their essential structure is about the same. We should make a culture of our material. Use a relatively low vessel whose walls are untransparent or may be made so by putting black paper around them, as lateral illumination is damaging to the plant. The vessel should be set in a bright place but be protected from direct sunlight. Into the river or spring water throw from time to time bits of turf boiled, and saturated with a nutrient liquid compounded as follows: To 100 cc. of water add 1 g. nitrate

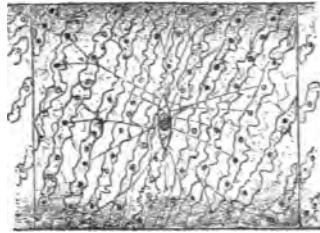


FIG. 74. *Spirogyra majuscula*. a cell of a filament shown by different focussing, also representing the central nucleus and its suspending threads. $\times 240$.

of potash, 0.5 g. common salt, 0.5 g. sulphate of lime, 0.5 g. sulphate of magnesia, a trace of finely pulverized phosphate of lime (11). *Spyrogyra* and other fresh-water algæ will flourish well under such conditions. The cells of the filament are, when full-grown, about $1\frac{1}{2}$ to 2 times longer than broad, Fig. 74. The cell membrane is lined with a delicate colorless protoplasmic layer which becomes clearly visible when the protoplasmic cell body is made to contract by withdrawing the water from the cell by means of glycerine, a solution of sugar, cooking salt, or saltpeter. To the protoplasmic layer succeed eight to ten chlorophyll

bands which appear to be wound steeply and closely about the cell. The bands are considerably indented, and are sufficiently transparent to allow us to look into the interior of the cell. Thick, round colorless bodies, amyllum centres, are embedded in the bands at irregular intervals. In these are the albuminous crystals and a surrounding layer of small starch grains. The crystals may be seen without reagents. They come out most distinctly when alcohol acidulated with picric acid is added at the edge of the cover-glass. Treatment with potassic iodide affects alike the color of the crystals and the starch membrane making the whole body a dark brown. The central nucleus is spindle-shaped in this species. Moved out of position by pressure it is seen to be disk-shaped from the front, so it in reality has the form of a biconvex lens. In its centre lies a large distinct nucleolus; sometimes two or even three of these may be seen in the nucleus. In other nearly related species the nucleus is thicker and in its natural position in the cell seems right-angled with the angles rounded off. The nucleus is surrounded by a very thin plasma layer from which delicate threads of protoplasm run out to the wall layer of the cell. The nucleus is suspended by these threads in the cell-sap which fills the cell. The threads spring from all of the sharp edges of the nucleus, mostly repeatedly fork in their course and fasten themselves to the inside of the chlorophyll bands particularly to the projecting places which cover the amyllum centres. This may all be seen, in most cases, by slowly changing the focus.

NOTES.

(1) H. Hoffman, *Icones anal. fung.*, 1—III; de Bary, *Morph. d. Pilze* etc. p. 49 ff.

(2) Ueber die Tafel in den Scheidewänden der Florideen, vergl. Bornet, *Etudes phycol.*, p. 100 und Schmitz, *Stzber. d. kgl. Akad. d. Wiss. z. Berlin*, 1883, p. 218.

- (3) Schmitz, Siphonocladaceen, p. 17; Strasburger, Zellh. und Zellth., III Aufl., p. 204.
- (4) Schmitz, Chromatophoren d. Algen, p. 37. See also, pp. 16 u. 35.
- (5) Nach Mittheilungen von A. W. Schimper.
- (6) Pringsheim, besonders in den Jahrb. f. wiss. Bot. Bd. XII, p. 294; A. Tschirsch, Ber. d. deut. bot. Gesell. Bd. I, p. 140. There the literature.
- (7) Fleming, zuletzt in Zellsubstanz, Kern und Zelltheilung, 1882, p. 379. There also the literature.
- (8) The capacity of the nucleus to absorb coloring matter with avidity was discovered by Thomas Hartig and published in Bot. Zeitg., 1854, Sp. 877, under the title of "Ueber das Verfahren bei Behandlung des Zellkorns mit Farbstoffen." Entwicklungsgeschichte, d. Pflanzkeims, 1858, p. 154. In animal histology the experiments of Gerlach should be quoted. Mikr. Stud. a. d. Geb. d. menschl. Morphol., 1858.
- (9) See Schmitz, Stzber. d. niederrh. Gesellsch., 13 Juli, 1880, Sep. Abdr., p. 2.
- (10) Strasburger, Zellb. u. Zellth., III Aufl., p. 173.
- (11) Nährstofflösung, nach Sachs Vorl. über Pflanzen-Physiol., p. 342.

LESSON XX.

DIATOMS, PROTOCOCCUS, YEAST, PROTOPHYTES.

THE diatoms or bacillaria are single-celled organisms which form a definite group by themselves, and occupy an intermediate position between animals and plants.* *Pinnularia viridis*, a species often occurring in standing or running water, gives us a most suitable object by which to examine the structure of the diatoms (1). The fresh-water forms attain considerable size and so generally give us a ready view of the structural relations of their organisms. With our highest available magnification, they appear either as an elongated ellipse or as a rectangular object with somewhat rounded corners. In the former case, we get a side view, Fig. 75 A, and in the latter a front view, Fig. 75 B.† In the side view we see the cell membrane marked by slender grooves which run from the edge toward the middle but not quite to it. They are mostly held to be flutings or depressions in the surface of the shell, thin places in the substance of the frustule. The middle, smooth surface left by the grooves has at its ends and also in the middle, strongly refractive thickenings, designated nodules. The two terminal nodules are connected with the middle one by a line which close to the central nodule bends aside both the same way and ends

*This statement will greatly surprise my readers, I imagine, coming from so eminent a botanist as Dr. Strasburger. That diatoms are plants, and plants too not of the lowest class, there is, I suppose, no good reason to doubt. Sachs places them among the *Zygosporæ* after the desmids. See *Lehrbuch d. Botanik, vierte Auflage*, p. 264, English Edition, p. 260. Thwaites first discovered the sexual reproduction of diatoms, by conjugation, forty years ago. See *Ann. Nat. Hist.*, 1847.—A. B. H.

† In general, diatoms are said to present a "front" view, when the side having the suture is turned towards us. The side view is when we look upon the broad side of the frustule.—A. B. H.

with a slight thickening. The terminal nodules are inclosed by the ends of these lines, which make a crescent about one side of them in the same direction as about the middle nodule. Between the nodules the lines widen a little as if they had an opening towards the inside of the cell. In a front view the grooves are seen only on the edges of the frustule. See *B*, Fig.

75. By focussing on the optical diameter of the cell and carefully observing the ends, we see that a middle strip of the wall is double. By a thorough examination we find that one part of the wall here shuts over the other. On the sides of the two elliptical parts of the wall which we saw in the side view, is fixed a membranous portion which ends in a free edge. The walls, then, of this cell, consist of two halves, one of which is inclosed in the other, and the whole cell resembles an elliptical box with a cover shutting down over the top of it. If we now pass from the optical diameter of our cell to the superficial view, we can follow the fine edges of the two halves of the cell, here, as delicate lines. In this



FIG. 75. *Pinnularia viridis*.
A, view of the side of the frustule. *B*, view of the front with the central surrounding band. $\times 510$.

genus it is easy to separate the two parts of the frustule by pressure or by chemical reagents. Examples may also be found in which the dead plants have more or less fully undergone this process. Pressure also will crack the frustule along a line parallel with the edge of the overlapping part, but a little distance from it. There

may, therefore, be two of these lines which may be supposed to be thin places in the frustule. They do not extend to the ends of the cell. The contents of the cell also have a different appearance in the two views. In the first, Fig. 75 *A*, a clear stripe extends through the middle of the cell from one end to the other. The colorless cytoplasm of the cell is consequently visible. Near the middle of the cell is a biconcave plasma-bridge. In this bridge is the nucleus provided with large nucleoli, not always visible without the help of reagents. Adjacent to the clear stripe on both sides are the endochrome plates, the brown-colored chromatophores, lying upon the overlapping parts of the frustule. In the plasma-bridge are slender rods, connected in pairs, of unknown significance. In the cell-sap are usually oil-drops of various sizes. In the front view, Fig. 75, *B*, the cell body appears uniformly brown because the chromatophores cover the whole colorless wall layer. At the extreme ends of the cell only may the colorless cell plasma be seen. The chromatophore is of uniform thickness and color throughout. In this view, also, the central plasma collection takes the form of a biconcave bridge.

If, now, we examine the *Cladophora* preparation, most likely we shall find some diatoms attached to that, which have been fixed and stained at the same time with the alga, and show the nucleus most beautifully.

We shall often find among *Pinnularia* many double compound forms. They are sister frustules produced by the self-division of the mother plant. The sides of the frustules adhere to each other, and if the walls of the two plants are already quite full grown, we shall find that the two outer halves of the two frustules shut over the inner halves. By the parting of the contents of the mother cells, these inner walls, for each daughter cell, are pro-

duced. Each cell possesses, therefore, an older and a younger wall, and the difference of their ages may be very considerable.

The *Pinnularia* specimens may be seen in motion. The cells proceed commonly in the direction of their longer axis, but may also sometimes turn aside from their path. They do not swim freely through the water, but creep over the surface of the substratum; probably the line which we saw in the middle of the frustule is a cleft in it, through which a protoplasmic membrane protrudes and becomes the organ of locomotion, a kind of pseudopodium. The motion is either uniform or by sudden movements.

If we place our *Pinnularia* preparation on a piece of mica and heat it red hot over a spirit or gas flame, and then examine it on a slide dry, and also under a cover-glass with high powers, we shall see that the diatoms are perfectly skeletonized. If the heat is applied but a short time, the frustules, from the burning of the organic substance, become brown, but by longer firing they are rendered colorless. Muriatic acid will not touch them. They consist of siliceous and show the finest characteristic of the structure of the cell wall. They must, therefore, be silicified in the highest degree. The grooves in this preparation show very distinctly as dark stripes; also other structural characteristics of the walls may be studied. Particularly beautiful are the lines, seen in the side view, running from the end to the middle nodules; they distinctly widen in the middle. In the front view the edges of the two halves of the frustule are very distinct, and the fine lines also parallel to these edges, but not extending to the ends. Beautiful skeletons of the diatoms may also be prepared by treating the living plants with a drop of concentrated sulphuric acid; then, after a little, adding first twenty per

cent and gradually concentrated chromic acid and finally removing both with water (2).

This remarkable construction of the cell wall out of two distinct parts is also common to other diatoms; so is also the power of locomotion. Even those forms which grow inclosed in gelatinous tubes have this power when once freed, but it seems mostly to be wanting in the thread-like forms. On account of the extraordinary delicacy of the structure of the cell walls of these plants, their frustulæ are used as objects for testing the higher powers of the

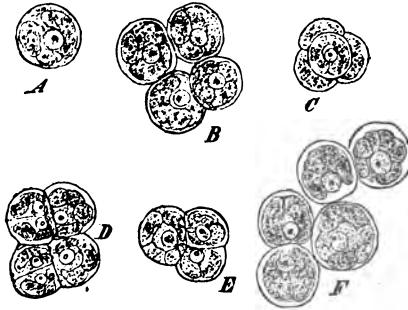


FIG. 76. *Protococcus viridis*, after treatment with potassium iodide of iodine. In *D* the cells to the left shortly after parting. $\times 540$.

microscope. *Pleurosigma angulatum*, when subjected to the highest magnification, shows the striæ resolved into regularly arranged hexagons.

We will study the *Protococcus* to learn the nature of the simplest form of the monocellular green alga. To this group belong all the green collections on the stems of trees, damp boards and walls and other such situations. We will leave the question in doubt whether our *Protococcus* is an independent species, or is only a single stage of development of another alga (3). The specimen, Fig. 76, which we took from an old trunk of a tree is

Protococcus viridis. Using a high magnification, we find it to consist of isolated, globular cells, or small groups of the same, Fig. 76, *A-F*. The contents of the cell are bright green, but the plasma mass is not uniformly colored, but by the highest magnification we discover a number of chromatophores, which, in mutual contact, occupy the surface of the cell contents. As their contact is not perfect, the colorless plasma comes in view between. Near the middle of the cell may be seen — not often however without the help of reagents — the nucleus with its nucleoli. The thin cell walls are colored violet with chloriodide of zinc. Most of the cells are seen in the process of cell division by means of a partition wall which halves the globular cells, Fig. 76, *D*. Neighboring cells divide in the same or in a direction at nearly right angles to the plane as the first. The daughter cells assume a globular form when they pass out of the original connection and either adhere for a considerable time or become fully separated, Fig. 76, *CF*. Treating the cells with potassium iodide of iodine will bring out the nucleus very sharply. The figures in the illustration are from an iodine preparation. Nucleoli will be clearly visible in every cell. In the newly formed cells the nucleus will lie on the side of the partition wall, Fig. 76, *D*. The iodine solution detects small starch grains in the chromatophores, but no amylum centres.

We meet a very simply constructed organism in the colorless fungus cells, hitherto included in the *Saccharomycetæ*. Take a small quantity of beer yeast from a well fermented mash in a brewery and examine it in water with a high magnification. Our field of vision will be filled with small cells which are the individual plants of the so-called beer yeast *Saccharomycis cerevisiæ* (4). The cells are globular or ellipsoidal, have a delicate cell-membrane, and

within one large or several smaller vacuoles and some granules which strongly refract the light, Fig. 77, 1. A nucleus exists but is not easily detected (5). In order to see it we shall have to treat the cells as in the case of the *Cladophora* with picric acid to harden them and then stain with ammoniacal hæmatein. It will then appear near the middle of each cell, small, round, and darkly stained. As we examine the living object we shall find some of the cells putting out one or more small buds from their sides, which gradually attain the form and size of the mother-cell and then separate from it. See Fig. 77, 2 and 3. In very energetic growth we shall find sometimes a number of the daughter cells connected together, forming a branched chain. In more gradual development each

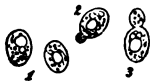


FIG. 77. *Saccharomyces cerevisiae*. 1, not budding, 2, 3, budding cells. \times 540.

new bud is separated from the mother-cell before another starts. This method of propagation gives them the name of *Saccharomycetæ* or budding-fungi. In solutions of sugar it produces an alcoholic fermentation. Recently (6) the specific independence of the *Saccharomycetis* has

been denied, and they have been explained to be gonidia or spores of different fungi, which possess the power in the right nutrient solution to go on reproducing themselves endlessly.

We will now turn our attention to a *Nostoc*, which on account of its symbiotic relations to another plant will be of interest to us. The latter plant is *Azolla Caroliniana*, cultivated in all botanic gardens. Since the *Azolla* is wintered in greenhouses we can obtain the *Nostoc* at all times. The *Nostoc* generally is very much inclined to live with other plants and we find it in very different ones, but principally as an element in the fronds of lichens. This fungus *Anabæna Azollæ* may be found on a particular part of

the plant. The leaf of the *Azolla* consists of two lobes; the upper one is fleshy and swims on the water, the lower is membranous and is immersed beneath the surface. On the inside of the upper leaf is a cavity which has an opening towards the interior of the leaf. This cavity is filled with the alga. From its walls grow branched hairs between which are the coils of the alga. In order to obtain the plant for investigation, tear away the surface of the leaf with a needle and lay a cover-glass on it, press on the glass a little and you will be quite sure to find the strings of the *Anabaena* on it. By examining the threads with a high power we find it to consist of a series of barrel-shaped cells, which are here and there interrupted by a larger ellipsoidal or round cell, the heterocyst, or terminal cell, Fig. 78. The threads are coiled about snake-like without visible jelly. The whole contents of the vegetative cells are verdigris-green, the terminal cells olive-green. Small dark granules are to be found in the contents of the cells but no nucleus. We often find the cells in the act of self-division, Fig. 78, *a* to *d*. Take a twig between the fingers and make a superficial section of the leaf and if the cavity of the leaf is cut just right we may see the *Anabaena* in its natural position—the jointed hairs with the alga coiled among them.

Quite of the same structure is the thread contained in the olive-green masses of jelly which one finds often in large quantities on the street, and which belong to *Nostoc ciniiflonum* (7).

In the investigation of those terrestrial forms of *Vau-cheria*, especially those which collect on flower pots, one

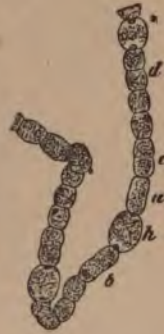


FIG. 78. *Anabaena azolla*. *a* to *d*, cells in successive stages of self-division; *h*, a terminal cell or heterocyst. $\times 540$.

will meet with the *Oscillaria*, which belongs to the self-dividing plants and is closely related to the *Nostocs*. It may be found in all standing water, on muddy soil and such like places. An unpleasant mouldy smell often betrays its presence. Cultivated in vessels it will creep up on the walls above the surface of the water. It consists of nearly straight or twisted threads, colorless or colored blue-green, or verdigris or olive-green to brown, which keep up a lively movement among themselves. The threads are free or inclosed in a gelatinous sheath. They may

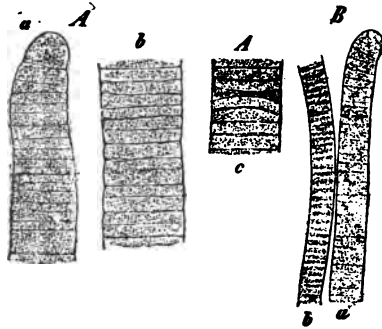


FIG. 79. *A*, *Oscillaria princeps*, *B*, *Oscillaria Fraelichii*. *a*, end of filament; *b*, a piece from the inner portion of the filament. In *B*, *b*, the granules are collected on the partition walls of the cells. In *A*, *c*, a dead cell is seen between the living ones.

occupy the sheath singly or in considerable numbers. The sheath arises from the outer membranous layer of the filament. Where these membranes have disappeared the sheath fails. The threads are divided into disk-like short cells by transverse division walls, the latter being seen easily in some cases and with much difficulty in others. With the exception of these differences a great uniformity in the structure of these organisms prevails. The contents of the cells are colored throughout. There is no nucleus, but numerous small grains appear, distributed uniformly,

or collected on the partition walls. It is a matter of indifference which species we select, but it will be of some advantage to take one with thick filaments, and visible partition walls like Fig. 79.

The movement of these plants is very interesting. With the thicker forms, having bent ends and distinct grains, and with a sufficiently strong magnification, the appearance can be fully examined. We see that the movement of the thread is connected with a gradual turning upon its axis. At the same time the thread exhibits irregular bendings which are the results of differences in the intensity of the growth of its different sides.

This bending may go on very slowly but may also give rise to a rapid motion when it is hindered by some obstacle, which being overcome the tension is suddenly relieved. The movements of the *Oscillaria* are forwards and backwards. It can take place only when the threads find some point of resistance.

The motion of the straight threads is the same as that of the bent ones, but is not so easily seen; for, in the former case, a single granule of the cell has to be fixed in the attention in order to see the motion of the thread upon its axis. The cause of this motion is not yet determined with certainty, but it has recently been asserted that it is produced by a protoplasmic process protruding through the membrane to the outside (8).

In the same class of organisms as the *Nostocs* and the *Oscillaria* are the still simpler formed *Chroococcaceæ* which we will study in the widely distributed *Glæocapsa* species. *Glæocapsa polydermatica*, Fig. 80, grows on moist walls



FIG. 80. *Glæocapsa polydermatica*. In A the self-division is beginning and in B at the left it is recently completed. $\times 540$.

or rocks and is distinguished by its dirty-green or olive-colored jelly mass, and the solid, distinct and repeatedly-laminated gelatinous covering. Another species with less beautifully laminated jelly inclosure would answer just as well. In all species, within the gelatinous envelope, are cells without nucleus, more or less distinctly granular and uniformly colored. By these characteristics of the cell body, these plants are distinguished from many forms of the *Protococcaceæ* which they outwardly resemble, especially the *Palmellaceæ*, for these have a nucleus and the chromatophores are separated from the rest of the cell plasma. *Glœocapsa polydermatica*, shortly before undergoing division, is almost globular, Fig. 80, *C*. It then begins to grow in length, and soon to show a contraction about the middle, Fig. 80, *A*, at which point a delicate division wall becomes visible. The daughter cells round themselves out and by the swelling of the division walls, and the thickening layer produced from them, they become widely separated. By the production of new gelatinous layers on the inside, the older outer ones become extended and at last burst and are thrown off (9). A great number of generations are consequently connected in one common cell family by the gelatinous envelope, in the rupturing of which the family is scattered. One sometimes finds a single cell existing alone by itself, in which case it is usually surrounded by a considerable number of jelly-layers, Fig. 80, *A*. In such a case the cell-division, not the wall-thickening, ceases.

In the *Nostocs*, *Oscillaria* and *Chroococcaceæ*, the cell-contents behave differently from what they do in all those plants heretofore examined. While we have previously found the protoplasm differentiated into cell-plasma, nucleus and chromatophore, we find here all these elements of the cell body united in one substance (10). Constant deviation in color from the pure green of the other plants requires that they be separately classed as *Phycochromaceæ*

or *Cyanophyceæ*. The low grade of their organization is indicated by their want of sexual reproduction. A kind of asexual reproductive system is peculiar to all these (together also with other asexual methods), namely: the one by vegetative self-division, hence, these organisms are called dividing-algæ, *Schizophyceæ* (11). Later investigations indicate that the thread-like *Schizophyceæ* are in a position to be separated into globular cells surrounded by gelatinous envelopes, that is, to enter upon the Glæocapsa-like condition. A corresponding behavior is observed already in the green algæ, in the *Protococcaceæ* and raises the question if *Protococcus viridis* be an independent species. This question is repeated in reference to the *Chroococcaceæ* which is perhaps only one stage in the development of the thread-like self-dividing algæ.

NOTES.

- (1) See Pftzner, in Hanstein's Bot. Abh., Bd. I, Heft II, p. 40 und Schenk's Handb. d. Bot., Bd. II, p. 410. In the first treatise, the literature may also be found.
- (2) Miliarakis, die Verkieselung, Würzburg, 1884.
- (3) See on this point especially Cienkowski, Bot. Zeitg., 1876, Sp. 17, u. Mém. biol. d. St. Petersburg, T. IX, p. 531.
- (4) Reess, Alcoholgärungspilze, 1870.
- (5) Schmitz, Stzber. d. niederrh. Gesell., 4 Aug., 1879, Spr.-Abdr. p. 18.
- (6) Brefeld, Bot. Unters. über Hefepilze, der Schimmelpilze, V Heft, 1883, p. 178.
- (7) See Thuret et Bornet, Notes algologiques, II, p. 102.
- (8) Engelmann, Bot. Zeitg., 1879, Sp. 49.
- (9) Schmitz, Stzber. d. niederrh. Gesell., 6 Dec., 1880, Sep.-Abdr., p. 7.
- (10) Schmitz, die Chromatophoren der Algen, p. 9.
- (11) See for example, Falkenburg in Schenk's Handbuch der Botan., Bd. II, p. 304.
- (12) Zopf, Bot. Centralbl., Bd. X, p. 32; zur Morpholog. d. Splatpf., 1882.

LESSON XXI.

SCHIZOMYCETES. USE OF THE IMMERSION SYSTEM.

FINALLY, we will examine some forms from the smallest organizations, the *Bacteria* (1) in order to become acquainted with the morphological relations there prevailing. We will not at first select any particular species for investigation, but will trust to chance for our specimens. Boil some green leaves of lettuce in a glass vessel and let it stand open in a room of relatively high temperature. In another glass vessel put some pease killed by steeping in boiling water and pour water over them. Distribute small fragments of cooked carrots, cabbages and potatoes about on watch glasses or object slides, putting them in warm, moist places, some in the open air and some under glass bells. On the lettuce decoction there will be a mouldy skin formed after two days; and on the fragments of the vegetable, small, whitish, rarely colored, masses of jelly. By putting a trace of this jelly in a drop of water on the slide and examining it with the highest possible magnification, we find a vast multitude of the very smallest bodies embedded in the jelly. They form bead-like series and may be found singly or in pairs or united into threads. This is a coccus formed of *Bacterium* embedded in jelly and is called *Zooglæa*. The jelly arises from the swollen membranes of the *Bacterium*. These membranes consist, in the putrefaction *Bacterium*, of a peculiar albuminous substance, mycoprotein, and of cellulose in the *Bacterium* not producing putrefaction. The *Bacteria* readily absorb aniline and azo coloring matter, and so we will stain these by add-

ing a little methyl violet, gentian violet, methyl blue, fuchsin or vesuvin to the preparation. Hæmatoxylin colors the jelly also, and we will use it only when we wish to bring that clearly into view. The gentian violet stains the *Bacteria* with the greatest rapidity and intensity. We see the *Bacteria* then very distinctly and can form a judgment as to their manner of increase, which is apparently by continuous self-division. This method of propagation gives these plants the name of the schizomycetes or "dividing fungi," in opposition to the "budding" of the yeast plant. Perhaps instead of these bead-like forms there are little rods in the jelly (see Fig. 83, *A*, farther on). By adding a solution of iodine to the preparation, the rods appear very distinctly to be a combination of short joints. The segments appear to be much shorter now than they did in the fresh condition; there appear also transverse walls which were quite invisible at first.

Certain *Bacteria* are distinguished by the fact that in their spore-forming stages they form a starch-like substance in their bodies which shows the blue or violet color when treated with iodine.

In the skin formed on the surface of the vegetable decoction occurs a form of *Zooglæa* (see Fig. 83 *A*). In this scum on the liquid, the cell-series become a superficially developed skin held together by the jelly. This is permeated with fine, wavy, elongated parallel filaments formed of micrococci or bacilli. The articulation of the micrococci and bacilli become very distinct by treatment with iodine solution. In such culture material as this we shall meet the swarming stage of development. We shall be especially sure to find it in one or two days in the pea water. The *Bacterium* will be found in rapid dancing motion, now forward, now backward, hastening in different directions. Sometimes fine cilia may be made out

as the cause of this motion, Fig. 83, *B*, and sometimes not.

If we investigate the scum on the lettuce decoction, after some considerable time we shall find the micrococci and the bacilli in a spore-producing state, Fig. 83, *C*. The cell contents of the bacillum will collect at one or more points and produce an ellipsoidal or roundish body, which afterwards becomes darker and is the "resting-spore." This is preserved, while the empty membrane of the cell finally is dissolved.

In other cultures we frequently find bacilli which produce resting-spores only in one end. Such forms are peculiar to the very widely distributed butyric acid ferment, *Clostridium butyricum*. Since the *Bacteria* are the smallest known organisms, it is necessary to employ our best lenses and best illumination in any thorough study of them. By this we mean the homogeneous immersion objectives and the Abbé illuminating apparatus. Still, in most cases, water-immersion lenses can be made to suffice. These lenses may be applied to the stand already described, but the Abbé illuminating apparatus cannot; for that we must have a larger stand.

The observer who works with a water immersion must have cover-glasses made of a definite thickness, corresponding to the correction of his objective system.*

If there is a screw-collar adjustment, the objective can be fitted to any thickness of cover-glass within permissible limits by means of the correction apparatus. On the Zeiss objectives, the figures are marked for each 0.01 mm. difference in the thickness of the cover-glass, and correspondingly on lenses of other makes. Put a drop of distilled water on the front lens of the objective, and take

*This would be necessary only for those lenses made without screw collar adjustment, as most American water-immersion lenses are not.—A. B. H.

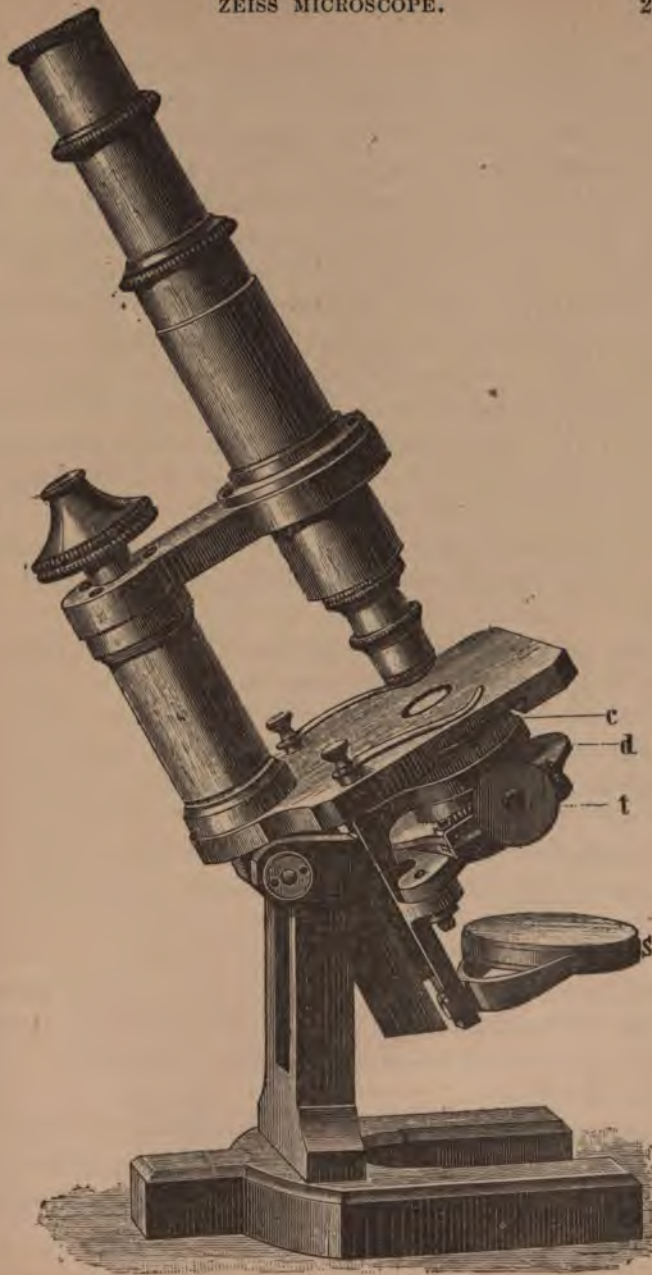


FIG. 81. Zeiss stand Va, $\frac{2}{3}$ natural size. The body may be inclined but not revolved. Abbé illuminating apparatus attached. *c*, condenser; *d*, diaphragm carrier; *t*, rack and pinion for the same; *s*, double mirror.

care that it does not dry out during the observation, but as it lies between the lens and the cover-glass it is in a situation quite unfavorable to evaporation and so will hold out generally for some hours. One must also be careful that in shoving the object-slide about he does not run the drop over the edge of the cover-glass and mix it with the fluid used in immersing the object for examination. If this should happen the objective should be immediately cleaned, and the drop of fluid on the cover-glass removed. If one does not know the thickness of the cover-glass used, the correction of the objective must be made experimentally during the observation; this is done by turning the adjusting collar back and forth till the place is found where the image is the sharpest and clearest. Since most of the objectives are made so that in this manipulation the front lens is immovable, the object remains clearly in focus.

The homogeneous-immersion lenses are without apparatus for correction, since the thickness of the cover-glass within allowable limits is a matter of indifference. A drop of the immersion fluid is put on the front lens of the objective. It may be cedar wood oil, or fennel oil with castor oil. The smallest possible quantity of the fluid should be used, since it will not evaporate, and will not need to be renewed during the observation. Care must be taken, as in the other case, not to let the fluid run over the edge of the cover-glass. A perfectly clean linen cloth should be used for wiping the objective. A piece of cloth moistened with chloroform may be used for the cover-glass. The homogeneous immersion lens will bear the use of the highest eyepieces.

In case one has a large stand like Zeiss, Va, Fig. 81, and an Abbé illuminating apparatus, the upper body of the stand should be swung back even farther than in the

illustration in order to affix the apparatus. This is done by removing the common mirror, and in place of the same putting in the whole Abbé apparatus, which consists of a condenser *c*, a diaphragm carrier *d*, and a double mirror *s*, all made in one piece. The condenser should be run back till the upper surface comes a very little below the upper surface of the stage as seen in the figure. As a rule use the plane mirror. The concave mirror should be used only with low powers when the field would not be uniformly lighted with the plane mirror. Diaphragms also should be used, and the narrowest that will give sufficient illumination. If it is desired to use a dark field diaphragm, turn the diaphragm carrier out to the right from under the stage and put the disk in and then push the carrier back to place. The rack and pinion, *t*, on the diaphragm carrier, serves to move the diaphragms out of the optical axis of the microscope, about which axis the whole also can be revolved in its mounting. By this means we get oblique illumination, but to this one seldom has recourse.

The Abbé illuminating apparatus is so convenient in use and possesses so many advantages in difficult investigations that it cannot be too highly commended. If one has a large stand with this apparatus, he should use it at all times even with the lowest powers; since, by the interchange and movement of the diaphragms, we may get any desired modification of illumination.

For dark weather and for the evening one needs a lamp with a large burner, and then, between this and the mirror of the microscope a large globe filled with a very dilute solution of cuprammonia may be placed. It will be an advantage to the eyes in working with the microscope at night to have the surrounding objects very nearly as brightly illuminated as is the field of the microscope.

The coloring substances already mentioned above for staining *Bacteria* should be prepared in an aqueous solution and should be used fresh, or at least freshly filtered. For this purpose one should have a saturated alcoholic solution always on hand and from this drop a little into a considerable quantity of distilled water; vesuvin, however, must be dissolved in water, as alcohol changes it, but should be filtered each time before using it. The *Bacteria* found in fluids should be spread in as thin a layer as possible on the cover-glass and then allowed to dry in the temperature of the room. If the fluid contains albuminous bodies or mucilage, these should be fixed, after being perfectly dried, by laying the cover-glass for several days in absolute alcohol, or simpler still, by subjecting it to a high temperature by passing it quickly several times through a gas or spirit flame, the surface of the glass containing the *Bacteria* being uppermost. The staining is done by putting a drop of the fluid on the cover-glass and letting it work for five or ten minutes, or by laying the cover-glass on a quantity of the fluid in a dish, and letting it swim there from ten to thirty minutes. Warming the fluid to 30° to 60° C. hastens the operation. After the staining, the cover-glass is rinsed in distilled water and dried by the heat of the room, after which, a drop of turpentine oil, xylol or cedar oil is applied, the cover-glass laid on a slide and the investigation begun. If it is desired to make a permanent mount of the preparation, the oil is removed and the preparation mounted in Canada balsam or dammar which has been dissolved in turpentine, not in chloroform. If the preparation is to be examined with a homogeneous immersion, care should be taken that the mounting fluid does not extend over the edge of the cover-glass, else the immersion fluid will come in contact with it and dissolve it and the whole surface of the cover-glass be besmeared

with it. To prevent this, a ring of asphalt varnish may be put on about the edge of the cover-glass, but not too far over the edge, by means of a camel's hair pencil.

Having stained one of the larger forms of *Bacteria* for investigation we may examine the cell contents by means of our highest objectives. We shall find it to consist of a homogeneous plasma in which are embedded fine or coarse granules which apparently consist of fat. No nucleus appears even in the largest forms. The body of the *Bacterium* is very seldom colored in a living state.

For the more definite investigation of the *Bacteria* we will take the smallest form, the round Bacteria of the pocklymph, *Micrococcus vaccinae* Cohn (2). Take fresh lymph and drying it on the cover-glass, stain as already directed with gentian-violet, when the small, round, dark-colored *Micrococcus* may be distinguished, singly or united in pairs. If fresh lymph be put under a cover-glass and protected from evaporation for several hours in a warm room, or better still, placed in a warm closet heated to 36° C., rosary-formed threads will appear, and after a still longer time heaps of the micrococci. These are also to be seen in the lymph preserved in glass tubes, where they are visible to the naked eye as small flocculent masses. These micrococci are introduced into the human body by vaccination, where they increase and produce the so-called kinepox, and by some unknown process give immunity from small-pox.

In water where decaying algæ are found—chiefly *Spirogyra* and *Vaucheria*—are very sure to be found also, thin, moving, screwlike forms, Fig. 82, flexible, twisted threads



FIG. 82. *Spirochata plicatilis*. After staining with aniline, the segments show bacilli. \times 510.

which move rapidly through the water. They turn upon their axes and bend here and there at the same time, sometimes suddenly stopping and then hasten on again. These organisms are in all probability *Spirochæte plicatilis* and belong to the swamp *Spirochæte*. When dried and stained as described above, it will be seen to be, not unicellular but to consist of successive joints, shorter or longer according to circumstances.

On the same decaying algæ, or on parts of other decaying water plants, or on other corresponding substrata, one frequently finds growing fine threads which belong to the *Beggiatoa alba* (3). These *Bacteria* are particularly distributed in water which receives the waste from factories, and in sulphur hot springs. They spread over the masses of mud at the bottom a dirty whitish covering. They belong to the largest *Bacteria* and may be examined with a relatively low power. The threads are of different thickness (from 0.001-0.005 mm.), are attached or free, the free ones being but parts of the attached. The threads are jointed, and the cell contents distinguished by strongly refracting granules. If we dry the preparation and then add sulphate of carbon the granules will be dissolved. They consist of sulphur. In filaments containing much sulphur the articulation is quite indistinct and can be seen only after staining with aniline or treatment with hot sulphate of soda or glycerine. In the hot glycerine the granules are partly, and in the sulphate of soda entirely, dissolved. By continuous transverse divisions the filaments may fall apart into micrococci and in the larger forms these may undergo a division perpendicular to that of the cells, dividing into quarters. Swarming micrococci, bacilli, and spiral forms have been observed among the *Beggiatoa*. The fixed filaments may be spirally bent in their upper parts. The straight and the spiral filaments alike exhibit

a creeping movement. The *Beggiatoa* disintegrates the sulphur combinations of the waters which it inhabits and thus causes a more or less considerable discharge of sulphuretted hydrogen.

Take now another form which unites the micrococci, the bacilli and the spirilli, and shows also the filamentous form. It is found in the white layer upon the teeth. Placed in a drop of water and examined with the highest powers there appear bacilli of various lengths, spiral-shaped *Spirochæta*, apparently unjointed filaments, and small closely compacted micrococci. Recently it has been demonstrated (4) that all these forms belong to the developmental stages of the same fungus *Leptothrix buccalis*, Robin. It lives as a saprophyte on the mucous membrane and on the covering of the teeth, but may under favorable conditions become a parasite, penetrate the tissue of the teeth and produce caries. Iodine solution will bring into view the bacilli which compose the long filaments, and the compacted micrococci are clearly resolved into their elements. These forms never fail and it is questionable if they always belong to the *Leptothrix*.

It may be said in general that the investigations of more recent times have shown that the different genera and species called from their outward form (5) *Micrococcus*, *Bacterium*, *Bacillus*, *Vibrio*, *Spirillum*, *Spirochæta*, etc., may belong to the morphological circle of one and the same species (6).

We shall use these terms only to designate and name given developmental forms, the micrococcus globular or ellipsoidal forms, bacillus filaments and spirillum the corresponding forms. The short rods will be called *Bacteria* to distinguish them from the longer bacilli; the simple filaments *Leptothrix*, and the branched *Cladothrix*; the screw forms, with relatively considerable diameter of wind and thickness of filament, *Spirilla*; or, if they have

little of the substance to be tested as thinly as possible on a cover-glass, letting it dry by the heat of the room; then fix the existing albumen by passing the preparation three or four times through a spirit or gas flame. Make a saturated aqueous mixture of aniline by shaking an excess of that body in water, and filter through paper previously moistened with distilled water, and to this add, drop by drop, a saturated alcoholic solution of fuchsin or methyl-violet till it begins to show opalescence. The cover-glass may now be floated on this fluid for a quarter or half a day or longer. The best effect is produced when the fluid is heated to 40° or 50° C., when the action need be prolonged not more than one-half an hour or an hour. Then rinse the cover-glass in water and lay it from three to five minutes in a solution of three parts nitric acid and 100 parts alcohol. This bleaches the whole preparation with the exception of the tubercle bacillus, if any are present. The preparation should be dehydrated with alcohol and examined in oil of cloves, the latter being removed with blotting paper, and the preparation mounted in dammar gum or Canada balsam. The preparation may be examined also in water. The stained tubercle bacilli may be seen with a magnification of about 300 diameters (9). Many other methods have been proposed for staining the tubercle bacillus, but only those which have some special advantages will be mentioned here (10). Let four grains of aniline oil be added to twenty-four grains 40% alcohol, which holds in solution sulphate of roseaniline or methyl violet, *BBBBB*. Dilute the solution one-half with distilled water. Filter and let stand, not too long. This fluid will stain the bacilli on the cover-glass, after which the preparation should be carefully washed with distilled water. If we desire to stain the substances containing the bacilli at the same time we do the plants themselves, it

must be done before the cover-glass is dried, by treating it with an aqueous solution of aniline blue, or with vesuvin or Grenacher's carmine. The tubercle bacilli will then be very sharply distinguished from the other *Bacteria* existing in the preparation at the same time.

This bacillus may be stained with methyl-violet alone if one will give the necessary time to it (11). Make a section of the tissue which has been hardened with absolute alcohol, or with chromic acid and then absolute alcohol, and place it in a small watch-glass, in a solution made by dropping four or five drops of the concentrated solution of methyl-violet in the glass full of water. The tubercle bacilli will be stained in from twelve to twenty-four hours, or in ten to twenty minutes by warming the fluid to 50° C. Wash the section in distilled water, lay it for five minutes in absolute alcohol, and fifteen or twenty minutes in a 1% acetic acid solution of Bismarck-brown, then again five minutes in absolute alcohol, then in oil of cloves and finally mount in Canada balsam which has no chloroform in it. The tubercle bacilli appear then as short rods colored an intense blue on a brown background. Other *Bacteria*, in case any exist, have lost their blue color and have become a more or less pronounced brown. The dry preparation on the cover-glass is colored much more quickly; with a strong saturated methyl-violet solution it takes a quite intense color in from half an hour to an hour at the temperature of the room. Then wash for one minute in absolute alcohol, and treat for five minutes with a concentrated Bismarck-brown solution. Rinse with water, dry, and mount as before. While the tubercle bacilli are but lightly stained in from half an hour to an hour in the methyl-violet solution in this case, the other *Bacteria* are immediately and intensely stained.

Bacteria found in other fluids may be double stained.

According to one of these methods (12) the fluid is spread upon the cover-glass, dried and fixed with the fumes of osmic acid, or with a 0.5% solution of chromic acid, wash and stain for half an hour to an hour with 0.001% aniline green, then again wash for twenty-four to forty minutes in slightly acidulated water to bleach the tissue. Then add for some minutes a weak solution of picrocarmine. Again wash the preparation and dehydrate with absolute alcohol or simply with drying; finally, when necessary, clarify with oil of cloves and mount in Canada balsam.

For the examination of *Bacteria* within the tissue, the latter should be hardened for a day or two in absolute or in 90 to 95% alcohol. Stain with gentian-violet or methyl-violet, and then bleach the tissue with strong alcohol with a trace of potash lye in it. Laying the preparation for a half minute in picric acid produces the same result and at the same time gives the tissue a yellow tinge. After bleaching the tissue with alcohol it may be again stained with iodine-green, methyl-green, eosin, magdala, acidulated fuchsin and other coloring substances for which the *Bacteria* have no affinity (13). A good double staining may be effected with gentian-violet and picrocarmine (14). For most cases a solution of gentian-violet in aniline water will give the best results (15). Prepare the latter as directed on p. 225 and dissolve in it dry gentian-violet to saturation or add to it a saturated solution of gentian-violet in alcohol, five parts to one hundred of the aniline water. Filter every time it is used. The solution may be kept for months. Immerse the section for some minutes in the solution, then from one to three minutes in a dilute solution of potassium iodide of iodine (one part iodine, two parts potassium iodide, and three hundred parts distilled water), then in absolute alcohol. The alcohol becomes a

purple-red and the section almost colorless. Clarify in oil of cloves and mount in Canada balsam, dissolved in xylol. The tissue appears colorless, the *Bacteria* a dark blue. Certain *Bacteria*, like the bacilli of typhus and the micrococci of many cases of pneumonia, are bleached by this process and so are thus distinguished from most other bacilli. An instructive staining may be got with safranin in sections hardened in alcohol or chromic acid (16). Mix like parts of saturated solutions of safranin in water and in alcohol and let the section lie for half an hour in the mixture, wash a little in water and some minutes in absolute alcohol, then transfer to oil of turpentine and mount in Canada balsam.

For the examination of *Bacteria* in tissue the Abbé illuminating apparatus may be used with the greatest advantage (17). But the diaphragms should be wholly removed, that the cone of light may be all utilized. By this means the image of the uncolored part will almost entirely disappear, while that of the colored, light-absorbing bodies will alone remain visible.

After having learned to distinguish the various developmental forms, and the various methods of investigation, we should next turn our attention to the methods of cultivating the various *Bacteria* so as to be able to produce any desired form and follow up its whole development. For this purpose we will begin with dry hay (18) over which we will pour a little spring water, and let the infusion stand for four hours in a warm closet at a constant temperature of 36° C. Then turn off the extract without filtering and dilute to a specific gravity of 1.004. Put the fluid in a retort holding 500 cm. and stop the mouth with cotton. Boil with a slight development of steam for an hour. Then keep it at a temperature of 36° C. In the course of a day, or a day and a half, there will be formed a delicate gray film

over the surface of the water, which consists of the zoogloea of *Bacterium subtilis*, the hay fungus or hay *Bacterium*. The characteristic of the spores of this *Bacterium* to resist a boiling temperature for a long time enables us to obtain a pure culture of it. The *Bacteria* generally are distinguished by this quality, their resistance to high temperature, but the hay *Bacterium* stands at the head in this respect. Transfer a portion of the film to the slide and examine with the highest powers; we shall find it to consist of long, jointed, wavy, parallel threads, which remain for the

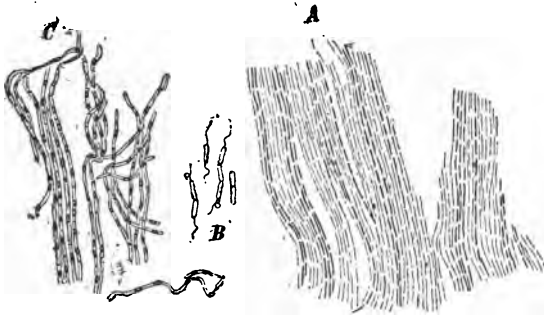


FIG. 83. *Bacterium subtilis*. A, film of mould. $\times 500$; B, swarming bacilli. $\times 1000$; C, forming spores. $\times 800$.

most part in their position because they are held together by an invisible jelly, Fig. 83, A. The filaments consist of little cylindrical rods, of different lengths, but which are generally two or three times longer than broad, the substance of which appears homogeneous, quite strongly refractive and colorless. The highest magnification shows nothing different. Chloriodide of zinc colors the whole mass of the bacilli a yellowish-brown very distinctly. This is the best of the iodine solutions to use for this purpose. The separate bacilli will appear shorter than in the fresh state but only because their terminations are all

clearly visible. In order to make the bacilli all quite distinct they may be stained in the ways already described with fuchsin, methyl-violet, gentian-violet, or vesuvin, and finally mounted for permanent preparations in Canada balsam or dammar. Sulphate of picric or picrate of nigrosine may be advantageously used for fixing and staining the preparation.

By using a magnification of 1,000 diameters we may see the dividing process of the bacilli direct (19). It is best to draw the portion of the filament under observation, at short intervals, by means of the camera, for the purpose of comparing the changes that take place in the bacillus. If there is sufficient nutritive matter in the fluid used in the observation, the bacillus will undergo the process of division in from half an hour to an hour and a half, and the higher the temperature of the room the quicker will the process be. The bacillus will increase in length without diminution in size till it reaches a certain point, then a dark division wall will appear in the middle, dividing the two halves of the bacillus from each other. This process of division will explain the arrangement of the bacilli and filaments, and it explains also the undulating course of the filaments, the intercalary growth taking place at all points, and the elongation of the filament, being more or less hindered, must cause the lateral sinuosity. The same cause also finally produces the folds in the film which are visible to the naked eye. We will now transfer a little of the film to a moist chamber to be examined in a suspended drop. Make the moist chamber in the simplest possible way by cutting from sufficiently thick paper board a rim no wider than the slide, and having the inner diameter of the same something less than that of the cover-glass to be used. Soak it in water and then lay it on the slide. Put a flat drop of the culture fluid with some of

the *Bacteria* on the cover-glass, turn it quickly over bringing the drop beneath, and lay it upon the pasteboard ring. If the observation is to continue long, the paper will need a drop of water from time to time to keep it moist. If the observation should be interrupted, the slide must be put in a larger moist chamber to prevent its drying up. If a definite place in the preparation is to be subsequently observed, the position of the slide may be outlined on the stage of the microscope, with a lead pencil, and thus the slide replaced again in its original position. When the nutritive substance in the fluid is exhausted the vegetative growth will cease and the formation of spores will soon begin. After six or eight hours, strongly refractive, ellipsoidal spores standing a little distance apart will appear, Fig. 83, *U*. The rest of the filaments will be empty, nothing but colorless envelopes connecting the spores. At certain points the spores will be found in the process of formation, and we shall see the strongly refractive substance collecting, in each bacillus, for the most part in the middle. The collection increases while the bacillus finally becomes empty and the spore is completed. In the course of a few hours the envelope becomes indistinct, and after the lapse of a day, the spores are set free and sink to the bottom of the drop. The spores are very darkly colored with gentian violet, and absorb other coloring matter like, but more intensely than, the bacilli. The spores germinate and grow very easily when introduced into other nutritive substances—slowly in the temperature of the room, more rapidly at 30° C. It is best to boil them for five minutes and then gradually cool, when the beginning of the germination will be seen in two or three hours (20). The spore membrane opens laterally and the new growth begins at this point and slowly elongates into a bacillus. The posterior end remains in

the spore membrane. About twelve hours will elapse before the bacillus will divide the first time. In the meantime the preparation will exhibit every stage in the development of the plant. For the most part one will see the sprouted bacillus soon set itself in motion, which introduces the swarming stage, in which condition it still bears the spore membrane with it on its posterior end. The number of swarming cells will constantly increase by successive self-division till they fill the whole fluid, before they begin to form the film. They finally collect on the surface of the fluid, come to rest and produce the film. The swimmers are of different lengths and consist therefore of a corresponding number of joints, Fig. 83, *B*. They move with an undulating motion. Put some of the fluid on the cover-glass and stain the swimmers as directed on page 220 (21). The swarm spores have cilia at both ends which are not easily seen (22).

Cultivation of *Bacteria* is usually carried on in small flasks (23). Many cultures may be undertaken on the object slide itself. Slide, flask and every utensil employed should be sterilized. This is done by passing them rapidly through the spirit or gas flame, or before the beginning of the experiment to lay them in absolute alcohol which quickly evaporates after taking them out. The nutritive solution to be used in the culture is boiled in the vessel, closed with a cotton stopper and covered with two thicknesses of blotting paper or linen. That boiling for a single hour is not always sufficient for the extinction of life in the *Bacteria* is shown in the case of the hay *Bacterium*. The adulteration of the culture usually comes not from the presence of other spores in the air, but from the failure perfectly to sterilize the utensils; and the danger which arises from an occasional opening of the vessel, for the purpose of introducing the spores, is far less than

that which comes from imperfectly sterilized vessels (24). Culture by the quantity for the purpose of obtaining pure material is conducted by different methods. 1st. The method by dividing the culture (25). This rests on the fact that if several of these forms are in the same nutritive substances, one of them will finally prevail over the others. When the culture is extended so far, a little is transferred to a second sterilized nutritive substance, and after a corresponding time from this to a third and so on. Thus there is the chance of getting at last a pure culture, and of that particular form which under the given conditions will reproduce itself the most quickly. 2d. The method of dilution (26). If the desired plant exists in vastly superior numbers this method produces mostly very good results. Dilute a little of the fluid containing the fungus in pure water, till there is probably not more than one plant in each drop of fluid. If now the plant sought for be by far the prevailing one, and a number of vessels with nutrient fluid be provided, and a single drop be put in each, the chance is very great that in some of them there will be the pure culture desired. 3d. The gelatine culture (27). Mix the nutritive fluid with gelatine so that at 30° to 35° C., it will remain fluid but stiffen at a lower temperature. For cultures that require a temperature from 30° to 40° C., the agar-agar, or sea-moss gelatine, is to be preferred as that does not soften at that temperature. A drop of the nutritive gelatine is spread out on the slide and allowed to stiffen there, and is then inoculated with the fungus by a needle dipped into the fluid containing it. The preparation is then set away under a water-closed culture-glass. The fungus will propagate there and will furnish us a ready means of observing all the stages in the history of its development, and give us material for comparison with the mass-culture. A stiff

gelatine has recently been made from the serum of the blood of cattle and sheep (28). It is obtained pure for the purpose of sterilization in test-tubes stoppered with cotton and daily for six days heated for an hour to 58° C., and then for several hours to a temperature of 65° C., till the serum stiffens. This amber-yellow transparent mass shares with agar-agar the advantage, that it can be kept at the incubating temperature.

One may judge of the purity of the culture in a mass by certain indications; as, for example, a uniform turbidness, or a uniformity of film on the surface, or of cloudiness at the bottom, finally a uniformity of color, or of gelatinous formation. Likewise, the purity of a culture may be assumed when it is preceded by an active fermentation or an intense putrefaction (29).

NOTES.

(1) For the statement following this see Zopf, die Spaltpilze; there the rest of the literature. For staining I depend principally on Hoyer, *Gazeta lekarska*, 1884. Apparatus for *Bacteria* culture are, according to R. Koch, furnished by Dr. Müncke in Berlin, Louisen Str. 58, and Rundorff, Berlin, Louisen Str. 47.

(2) Cohn, *Beitr. d. Biol.* Bd. I, p. 161; Zopf, l. c., p. 92.

(3) Engler, Bericht der Commiss. zur Erf. d. deut. Meere, 1881; Zopf, d. Spaltpilze, p. 13, 75 ff. There also the literature.

(4) Zopf, same work, p. 80.

(5) Cohn, work quoted above Bd. I, p. 125.

(6) See the literature in Zopf, die Spaltpilze, 1883.

(7) Zopf, l. c., p. 5.

(8) Von R. Koch, *Berliner Kleinische Wochenschrift*, 1882, p. 221.

(9) See Friedländer, *Mikr. Technik*, II Aufl., p. 53.

(10) Von Ermengem, *Bull. d. séances d. l. Soc. belge de Microsc.*, 29 Juillet, 1882, p. CLI.

(11) Baumgarten, *Zeitschr. f. wiss. Mikrosk.*, Bd. I, pp. 53, 54, 57.

(12) According to Soubbotine, *Arch. de phys. norm. et path.*, T. XIII, 1881, p. 477.

(13) According to Hoyer l. c.

(14) Weigert, *Virchow's Archiv*, Bd. LXXXIV, p. 201; Firket in Bizzozero's *franç. Uebers. des Manuel de Micro. clin.*, p. 314.

- (15) Gram, Fortschr. d. Med. 1884, p. 185.
- (16) Victor Babes, Archiv f. Mikr. Anat., Bd. xxii, pp. 359 und 361.
- (17) Introduced by R. Koch; Unters. über Aet. d. Wundinfektionskrankheiten, Leipzig, 1878.
- (18) According to a method recommended by Roberts and Buchner. See Zopf, die Spaltpilze, p. 57, upon which work I have generally depended for the literature.
- (19) See Brefeld, Schimmelpilze, Heft iv, p. 38.
- (20) See Brefeld, p. 43.
- (21) See Koch in Cohn's Beiträg, z. Biol., Bd. ii, p. 402.
- (22) Brefeld, p. 40.
- (23) Buchner, in Naegeli's Unters. üb. niedr. Pilze, p. 192. There also illustrations of the vessels used.
- (24) Buchner, Stzber. d. bair. Ak. d. Wiss., 1880, p. 381, u. in Naegeli's Unters. über niedr. Pilze, p. 159.
- (25) Introduced by Klebs, Arch. f. exper. Path., Bd. i, p. 46; Zopf, p. 43 ff.
- (26) By Naegell, Stzber. d. kgl. bair. Ak. d. Wiss., 1880. p. 410, u. Unters. über niedr. Pilze, p. 13; Buchner, Stzber. d. kgl. bair. Ak. d. Wiss., 1880, p. 374 and in Naegeli's Unters. über niedr. Pilze, p. 146.
- (27) Introduced by Brefeld. See Schimmelpilze, Heft i, p. 15.
- (28) Koch, Zur Untersuchung pathog. Organismen, Mitth. aus dem kgl. Gesundheitsamte, 1881, p. 18.
- (29) According to Zopf, l. c., p. 44.

LESSON XXII.

THE REPRODUCTION OF THE ALGÆ.

AFTER having taken a survey of the general field of morphological inquiry in respect to the higher and lower forms of plant life, it shall be our task to solve by microscopical investigation some of the more important problems involved in their special morphology. We shall follow a course the reverse of that heretofore pursued, and proceed from the simplest to the most highly organized forms. We have made a beginning already in the last lesson, with the *Bacteria* whose entire development we have examined. We conclude now with a consideration of the sexual and asexual processes of reproduction in the algæ.

We often have an opportunity to observe the conjugation of the *Spirogyra* (1). The plant may be known by the crisp appearance and the closely attached filaments of the mass. The process may be easily followed. If one does not wish to put the plant on the slide under a cover-glass, he may use the suspended drop in the moist-chamber, as described on page 230, for studying the plant. The conjugation in most of the species takes place by the formation of a bridge, or connecting passage, between the cells of two filaments lying near each other. Short blunt projections appear on the contiguous sides of the cells, which finally touch and unite and form the connecting tube. In many cases it is possible to distinguish the male from the female filament before the act of conjugation by the swelling of the cells into a barrel-like shape. After the conjunction of the two lateral processes the contents of the

male cell first begin to round themselves up and separate themselves from the cell wall on all sides; then pass into the connecting tube and through the dividing wall of the same. The female cell has, in the meantime, gathered its contents together upon the entrance of the contents of the male cell. Both cells participate in the contact. Their contents are mixed. The chlorophyll bands commingle. The two nuclei are united and form one, but this can be seen only by straining the filaments (2). The body thus formed soon begins to contract and in the course of an hour its interior cavity has entirely disappeared. It is called a zygospore. The chlorophyll bands are pressed inward and the exterior is occupied by a colorless frothy protoplasm. After the lapse of twenty-four hours it again increases in size. A space appears in the interior and the whole body becomes ellipsoidal. The chlorophyll bands return to the exterior and a distinctly outlined double membrane covers the spore.

This method of conjugation is characteristic of this whole group of algæ, to which belong also besides the *Spirogyra* the *Zygnema* species, so widely distributed in fresh water, and recognized by two stellate chromatophores in each cell; and the desmids so prettily formed. Nearly related to the latter are also the diatoms in which occurs the typical conjugation process.

The *Cladophora*, whose structure is already known to us, furnish a very favorable object in which to study the swarm-spores (3). It is to be regretted that it is not always inclined to form swarm-spores. It is relatively easy to get them in the marine forms, by putting the plant in a large vessel of sea-water. Still, if our fresh-water form, *Cladophora glomerata*, when taken from rapidly flowing water, be laid in a shallow dish with the water not over 1 cm. deep, towards evening, the swarm-spores will most

likely appear by the next day. The formation of the spores begins at the end of the branches and extends towards their base. Thus it is easy to observe all stages in their development, at the same time. Beginning with an unaltered cell at the base we look along toward the top of the branch. We first notice the well-known structure and observe all that can be seen without reagents: the polygonal, closely-aggregated chromatophores which bear the small, pale starch grains, and in part also the greater *amylum* centres; the plasma plates which run through the cell cavity and contain in part also the chromatophores. If, now, we move gradually along towards the spore-forming cells we shall notice first a change of color in the cell contents. With a sufficiently high magnifying power we shall observe that the *amylum* centres have disintegrated into single starch grains and the chromatophores have also divided into smaller bodies. The next stage shows the chromatophores arranged in a reticulated order so that the colored contents of the cell which surrounds the cell cavity seem to be separated into polygonal sections of nearly the same size. The middle of these sections seems to be free from granules, and if we fix and stain them we shall find a nucleus at that point. The membrane which incloses the whole cell contents becomes thickened and easily visible. At a point near the forward end of the cells, and in terminal cells quite at the extremity, a colorless lenticular mass of protoplasm is to be seen. The cell membrane swells and is arched up, at a point corresponding to the middle of this collection, and by reason of the swelling and consequent increase of volume papillate projections appear. The next change consists of the drawing of the chromatophores toward the centre of the polygonal section and the consequent separation of the latter by clear boundary lines. This is followed by the rounding

up of the sections and their separation from each other. The peripheral layers appear like protuberant knobs. The outer membranous layer formed from colorless protoplasm takes no part in this differentiation of the chlorophyll-bearing contents of the single sections, but changes into a colorless mucilage which plays an important part in the discharge of the swarm-spores. Corresponding to the thick collection of colorless protoplasm at the subsequent place of exit, is the mass of formed mucilage, here the greatest, and the still coherent mass of swarm-spores remain at this place correspondingly removed from the swelling cell wall. In the mulberry-shaped mass of swarmers the cylindrical inner cavity may be seen; but in case the spores are very plentifully developed, this may be lacking, but it commonly exists and the spores form two or three layers about it. The spores are pear-shaped. The forward pointed colorless end may be easily distinguished from the rounded posterior end containing chlorophyll. At the front end is a red spot called the "eye speck." The cell-membrane is so much swollen at the place where the papillæ are that its outline can scarcely be made out. By a continuous observation, one will see the beginning of the emptying out of the swarm-spores. By the pressure of the swelling cell-contents the papillæ will be ruptured, and the spore mass will be thrust violently out. Likewise the finely granular contents of the cell cavity will come out with the spores, the latter after a while setting themselves in motion. The contents of the sporangium show a diminution of mass, draw back from the cell wall, the gelatinous mass which pressed upon the cell contents apparently lying against the wall. If any of the spores remain in the sporangium they soon set up a movement among themselves and one after another escapes through the papillæ. Some remain

behind. If one examines the object in a suspended drop, he will find many of the spores collected on the side towards, or opposite to, the window, under the influence of light, but those which are not sensitive to light continue to swim about for a long time in an indefinite path, and gradually, with the diminution of their energy, reach the edge of the drop, where they come to rest. There they are rounded up and covered with a cell membrane. The spores are very well fixed with a little potassium iodide of iodine, Fig. 84. Two cilia are seen (four in some species of *Cladophora*) to spring from a projection on the anterior end of the spore. If the spore lies in a favorable position an application of the iodine solution will reveal a small nucleus in its forward colorless end—see the figure—the nucleolus being for the most part very distinctly stained.



FIG. 84. *Cladophora glomerata*. Swarm-spore fixed with potassium iodide of iodine. On the right side is seen the eye speck. And in the colorless anterior end the nucleus. $\times 540$.

These swarm-spores are asexual, but the *Cladophora* produces other smaller spores which are sexual and conjugate with one another. The latter have thus far been found only in marine plants (4).

From the order of *Siphonaceæ* we select *Vaucheria sessilis* for the study of the formation of its swarm-spores and sexual organs. If we collect a stout specimen from standing, or better still from flowing, water and lay it in a flat vessel with fresh water, we may quite confidently reckon on a number of swarm-spores the next morning. They will be all the forenoon discharging, so we may easily find all wished-for stages of development (5). If we look over the whole plant with a magnifying glass we shall easily recognize the first beginnings of the sporangium in the darker color of the ends of the filaments. When a group of the filaments is found which

appears to furnish the desired condition, it should be transferred by the forceps without injury from its place of growth to the object slide, where the further development may be directly studied. To obviate any interference which the pressure of the cover-glass might exercise upon the processes of development it is well to put a fragment of pith or horse hair under one edge. If a sporangium

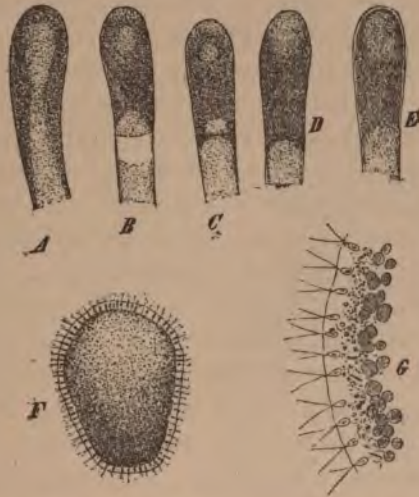


FIG. 85. *Vaucheria sessilis*. *A* and *B*, beginning of the sporangium; *C* to *E*, formation of the swarm-spores from the contents of the sporangium. *A—E*, $\times 95$. *F*, a free swarm-spore. $\times 250$. *G*, a piece of the outer colorless plasma layer which occupies the anterior end of the swarm-spore. $\times 450$.

forms in the end of a branch, the contents, rich in chlorophyll, gather themselves together and the cell swells out into a club-shaped form. The cavity within begins to narrow, Fig. 85, *A*, and soon the upper part is separated as a spherical vacuole. Then the sporangium is set off from the rest by a division wall, by the formation of which the contents of the sporangium are separated from those of the

rest of the frond leaving a clear space between, Fig. 85, *B*. A clear border is formed about the contents of the sporangium which soon takes on a radial structure, *E*. The border consists of colorless protoplasm. The radial structure arises from the cell nuclei which gradually collect here and are placed in this position, *F*, *G*. These nuclei are to be seen only by the use of proper reagents and the highest magnification (6). When the swarm-spore is ready, it must be set free. This is done in the following manner. The sporangium is torn with a jerk and in the same instant the anterior part of the swarm-spore springs out through the opening and begins at the same time to rotate on its longer axis. Its passage out through the opening occupies somewhat over a minute. Sometimes the forward part of the swarm-spore is twisted off from the rest which remains in the sporangium, the result being that each part forms a perfect though smaller swarm-spore. This is made possible only by the fact that the spore contains many nuclei and so there is the one necessary for each part. The movement of the swarm-spore continues for about a quarter of an hour, the direction of it not being influenced by the light. The spore is oviform, the larger end forward, which contains the cell cavity. The cilia, which cover the whole body as a short down, are seen only at the moment when the spore comes to a rest, for in the next moment they are withdrawn into the body of the spore, which shows during this process a wrinkled surface. After that, the surface becomes smooth. During the withdrawal of the cilia, a thin membrane is seen to be formed about the spore, which now rounds up, the colorless border disappearing, the chlorophyll grains returning to the surface and the cell wall becoming rapidly thicker.

In the terrestrial form of *Vaucheria sessilis*, the sexual organs are easily found; the pistillate organ, the oögon-

nium, being attached immediately upon the thallus filament, the antheridium on the other hand terminating a branch bent like a ram's horn and growing immediately from the thallus filament. The two usually form a pair near each other; sometimes one antheridium is seen placed between two oögonia. One should select plants of this species for observation, and not those of *Vaucheria terrestris* often found on moist ground, for here the oögonium and the antheridium are set on a common lateral branch. The *Vaucheria sessilis*, living in water, forms in culture the swarm-spores already studied, and after a few weeks produces sexual organs also.

The oögonium, Fig. 86, *o*, is obliquely egg-shaped, filled with plasma containing oil and chlorophyll, and separated from the thallus thread by a division wall somewhat above its place of insertion (7). The oögonium is provided with a lateral bill-shaped out-

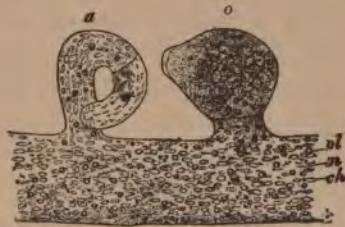


FIG 86. *Vaucheria sessilis*, piece of the thallus with reproductive organs. *o*, oögonium; *a*, antheridium; *ch*, chromatophore; *ol*, oil drops; *n*, nucleus, seen only when properly stained. $\times 240$.

growth in which colorless protoplasm is collected. The latter occupies the whole upper third of the oögonium in some stages of its development. By a continuous observation of such an oögonium, we see the colorless contents at the end send out a papillate process which slowly rounds out into a sphere and separating from the oögonium slowly sinks to the bottom of the water. This observation teaches not that the membrane at the end of the oögonium is perforated, but rather that it swells into a jelly-like envelope and the drop of plasma is pressed out through the gelatinous substance. The remaining contents of the oögonium round up, its colorless end being the germinal vesicle.

The branch bearing the antheridium is more or less bent, its upper third is set off from the rest by a division wall and becomes the antheridium, Fig. 86, *a*. In its ripe state it is distinguished by its colorless contents, while the branch which bears it is rich in chlorophyll grains. The apex of the antheridium is usually turned away from the oögonium. In the colorless contents of the antheridium, short rods longitudinally arranged may be more or less clearly distinguished. At the time when the oögonium exudes a part of its plasmatic substance, the antheridium opens at the apex and discharges its mucilaginous contents. The greater part of it remains in the form of colorless bubbles in the surrounding water where it slowly disorganizes. A smaller part assumes the form of very minute spermatozoids. These lively swarming spermatozoids soon collect on the gelatinous mass at the end of the oögonium. Some penetrate to the colorless embryo-sac of the spore, and in favorable cases may be seen to commingle with that. After a short time the fertilized spore — oöspore — will be surrounded by a delicate membrane which may be seen with special distinctness at the embryo-sac. In the space of a few hours the colorless protoplasm is distributed uniformly through the oöspore. Older spores are filled with large oil drops, show a brown spot on the inside and possess a hard cell wall. If one fixes the moving spermatozoids with potassium iodide of iodine it will be found to be provided with two laterally-inserted oppositely-arranged cilia of unequal length.

NOTES.

- (1) de Bary, *Conjugaten*, p. 3; Strasburger, *Befr. und Zellth.*, p. 5; Kny, *Wandtafu*, Text, p. 11.
- (2) Schmitz, *Stzber. der niederrh. Gesell.*, 4 Aug., 1879, p. 23.
- (3) Thuret, *Ann. d. sc. nat. Bot. III Sér.*, XIV T., p. 219, and

Taf. 16; Schmitz, Siphonocladaceen, p. 34, u. Chromatophoren, p. 119, Anm.; Strasburger, Zellb. u. Zellth., III Aufl., p. 72.

(4) See Areschoug, *Observ. phycol.*, II, *Acta soc. scient. Upsal*, Vol. IX, 1874.

(5) Thuret, *Ann. d. sc. nat. Bot.*, 2 sér., Bd. XIX, p. 270; Strasburger, *Zellb. u. Zellth.*, III Aufl., p. 213, u. 84.

(6) Schmitz, *Stzber. d. niederrh. Gesell.*, 4 Aug., 1879, *Sep. Abdr.*, p. 4; Strasburger, work before quoted, p. 88.

(7) See Pringsheim, *Monatsber. d. kgl. Ak. d. Wiss, zu Berlin aus dem Jahr 1855*; de Bary, *Ber. d. Freib. Naturf. Gesell.*, 1856; Strasburger, same work quoted, p. 90.

LESSON XXIII.

REPRODUCTION OF THE FUNGI.

IF one puts a piece of moist bread under a glass bell, in a few days it will be covered with a thick mat of fungus filaments which belong to the *Phycomycetæ*, *Mucor mucedo* (1). It grows very luxuriantly on fresh dung kept in a close moist place. Its fruiting filaments rise above the substratum several millimeters high, turn towards the source of light, and are terminated each by a round, yellow or brown, minute bead which may be easily seen with the magnifying glass. By transferring some of the plant to a drop of water on the slide and sufficiently increasing the magnification, it may be demonstrated that the mycelium consists of thick, much-branched irregularly-divided tubes, out of which arise these straight undivided and unbranched filaments which bear the spherical sporangia at the top. Those which are unripe preserve their form in water and have a yellow-brownish protoplasm. In the youngest stages, the fruit stem is not marked off from the sporangium, but further on a division wall arched strongly outward is produced on the inside of the sporangium, so that the fruit-stem ends in the sporangium, in a so-called "columella," a club-shaped process. The ripe sporangia are disintegrated in water, only small fragments of the wall remaining, formed of fine needles, which consist of the oxalate of lime (2). The freed spores lie nearly at a uniform distance apart embedded in a colorless mucilage as may be demonstrated by moving the cover-glass. Beneath the columella is a small collar which constitutes the remainder of the calcareous

incrustation. In the wall-lining of the fruit-bearing filament, if it be not too old, one may follow the longitudinally running streams of protoplasm. *Mucor* mycelium are polynucleated, the nuclei very small and seen only by staining. On the manure culture the fungus occasionally, yet rarely, develops zygospores which appear as black points. They are produced by the conjugation of the club-shaped ends of the hyphæ or mycelium thread. On the ripe, dark, warty zygospores one may see the two mycelium threads as clear circumscribed circular spots.

The cause of the potato blight is a *Phycomyceta*, the *Phytophthora infestans* (3), whose germinating filaments penetrate through the epidermal cells into the intercellular spaces of the leaf, and ramifying there destroy the tissue of the leaf, forming brown flecks on the surface which constantly increase in size. In order to obtain the plant in a fruiting state in a larger mass, put a blighted branch of the plant under a glass bell, the air in which is saturated with vapor of water, and let it lie there for two days. The blighted leaves soon become covered, especially beneath, with a white mould, which is formed of the filamentous fruit-bearers, spore-stalks, of the fungus. This mould is particularly well developed on the edges of the brown spots. A superficial section shows us that the spore-bearing filaments grow out of the widely opened stomata. This fact may be observed indeed, though not so satisfactorily, by using a piece of the leaf of full thickness. These conidia-bearing filaments appear to be delicate, unicellular threads filled with finely granular protoplasm and branched at top, Fig. 87, *A*. The branching is monopodial, and the number of branches but two or three, which have irregular swellings along their course.

The conidia-bearing filaments in dry air collapse and twist about on their axes. Sometimes we find on the end

of the branches spores in the process of development, but the ripened citron-shaped spores always fall off when the preparation is put in water. To find the ripe spores *in situ*,

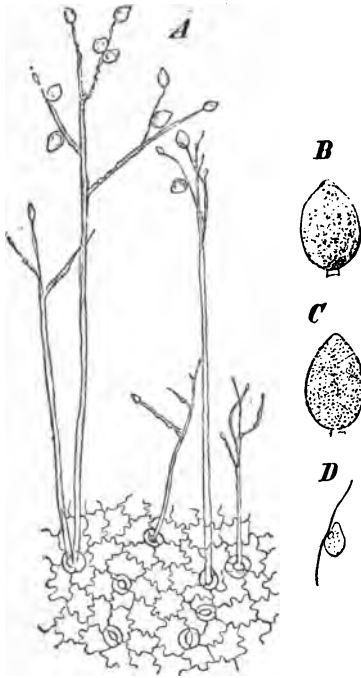


FIG. 87. Superficial section of the epidermis of leaf of *Solanum tuberosum*, out of the stomata of which are growing the conidia-bearing filaments of *Phytophthora infestans*. $\times 90$. B, ripe conidia; C, one with the contents divided; D, a swarm-spore. B-D, $\times 540$.

the plant must be examined dry, but even then a slight trace of water should be introduced under the cover-glass, for the plant rapidly shrinks up when dry. Specimens collected in the open air produce the conidia-bearing filaments only on the under side of the leaf. The filaments are not so long as those produced in the moist chamber and therefore not so easily seen with the naked eye. A cross-section through the leaf at the border of the fleck, made by means of elder-pith, will enable us to follow the course of the filament in its exit through the stoma. Frequently, also, several hyphæ will collect and branch at this place and send up a number of

spore-bearing filaments. By following out the course of the hyphæ in the tissue of the leaf, we shall find that it runs in the intercellular spaces. *Phytophthora* is distinguished from the nearly related *Peranospora* species by

forming but few and short processes for absorbing the juices, among the cells of the host plant so that one often looks in vain for them. The delicate mycelium threads, on the contrary, cling fast to the cells of the host. The chlorophyll grains of such cells first become brown and then they with the other elements of the cell contents dissolve and mingle and run together in a brown mass, and finally the whole cell collapses. The spores are citron-shaped, Fig. 87, *B*, somewhat pointed, with short stems and finely granular contents. The membrane at the apex is very delicate and a little swollen. The spores are produced as we have seen on the ends of the branches of the conidia-bearing filaments, but when they are fully grown, the end of the branch grows out beyond the spore, presses it over to one side so that it stands nearly at right angles with the stem and finally at the end produces a new spore. See Fig. 87, *A*. By sowing the spores in a drop of water on a cover-glass, and being careful to get the spores immersed in the water, and suspending the drop by laying the cover-glass on a small moist chamber, in a shaded place, we shall have, in the course of an hour or more, the beginnings of the swarm-spore forming process. Since the swarm-spores are formed from the contents of these larger forms we call them conidia and not spores. Among the conidia are sporangia which behave like common spores, for we see some on the edge or surface of the drop of water which put out a germinating tube from the forward papillæ. In the immersed spores the contents are divided into an indefinite number of cells, *C*, which show in each a small central vacuole. The apex of the conidium soon swells and finally dissolves leaving a small orifice through which the masses of differentiated contents are pressed out one after another. They speedily become swarm-spores. By fixing the swarm-spores with iodine

solution we recognize two cilia inserted laterally on the spore in the neighborhood of the vacuole, *D*. The swarm-spore continues to move for half an hour. It then comes to rest, surrounds itself with a cellulose membrane and soon puts out a germinating tube. This germinating tube from a swarm-spore or from a conidium direct is what penetrates the epidermis of the stem or leaf of the potato, and so infects a perfectly sound plant. By the formation of conidia the rapid increase of the fungus is provided for.

Sexual reproductive organs have not yet been discovered in this species, though they are well known in the nearest related *Peranospora* species. Branches of mycelium swell mostly at the ends, forming a spherical mass within the tissue of the host plant, which is separated from the mycelium filament by a division wall. It is called the oögonium. On each oögonium there lies the end of a mycelium branch, which has been differentiated as an antheridium. The greater part of the protoplasm of the oögonium collects into a central spherical egg, into which the antheridium thrusts a fertilizing tube, whereupon it surrounds itself with a thick membrane.

Upon almost any moist object, which has the least trace of nourishment in it for the fungus, may be found the blue green mould, *Penicillium crustaceum* Fries (4). It is the most widely distributed of all the moulds and may be found in all sorts of places. As convenient a way as any to obtain specimens for examination is to moisten a piece of bread and put it under a glass bell. *Mucor* will first appear, but will be gradually displaced by *Penicillium* which will spread a blue-green cover over the substratum in about eight days. The color comes from the spores but only when they occur in large quantities. Examine a little of the material in water. The mycelium consists of branched multicellular hyphæ, the cells separated by

transverse walls. The immediately visible contents are finely granular protoplasm with small vacuoles. Single filaments not distinguishable from other mycelium filaments have formed fruit-bearers. On their tips is a whorl of short branches, Fig. 88, *s'*, which either themselves bear whorls of basidia, or whorls of short lateral branches which do bear the basidia. This manner of branching gives to the fruiting filament the appearance of a hair pencil. Frequently also secondary pencils spring from beneath a division wall of the primary filament. See the figure. By a sufficiently high magnification we shall discover that the basidia are cylindrical, prolonged at the end into a finely pointed process, called the sterigma, *st*. This sterigma swells and rounds at the end forming a rapidly growing spore. Beneath this is a second swelling which forms a second spore and so the chain of spores is produced. The terminal spores are thrown off while those below are being produced. *Penicillium* tufts fixed with absolute alcohol may be easily colored with hæmatoxylin, after which it will be seen that in the cells of the mycelium and

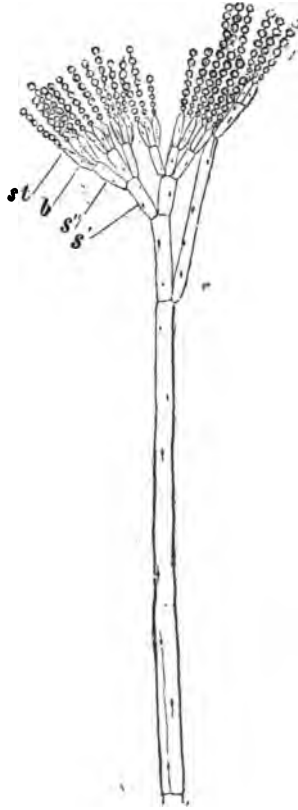


FIG. 88. *Penicillium crustaceum*. Fruit bearer with branch whorls, *s'* and *s''*; basidia, *b*; sterigma, *st*, and spores. Nuclei visible. From an alcohol-hematoxylin preparation. $\times 540$.

of the spore-bearing filaments, numerous nuclei occur (5). They are so small as to require the highest magnification. They are elongated in the direction of the longer axis of the cell and connected by fine plasma strings. In the long cells there are several, in the short cells of the whorl on the aërial filament but one or two, in the basidia but one at the upper end. But the basidia are commonly so filled with contents at their apex that it is almost impossible to make them out. With the strongest magnification one may detect a nucleus in each of the spores.

Other fruit bodies than these under consideration have been observed in *Penicillium*. They are produced in certain cultures, have the size of a small pin-head, and are of a yellowish color. After a long resting period, they form asci within, each of which produces eight spores. This places the *Penicillium* among the *Ascomycetæ*, and indeed as the representative of that division of the Cleistocarp *Ascomycetæ* with closed fruit bodies. From the spores produced in the asci, the pencil-like fruit-bearing filaments may be cultivated on the object-slide.

NOTES.

(1) Brefeld, Schimmelpilze, Heft I, p. 10. There also the literature.

(2) Brefeld, l. c., p. 18.

(3) See de Bary, Ann. de. sc. nat. Bot., IV sér., p. 32, und Beiträge zur Morph. u. Phys. der Pilze, Heft II, p. 35.

(4) Brefeld, Schimmelpilze, Heft II.

(5) Strasburger, Zellbild. u. Zellth., III Aufl., p. 221.

(6) Brefeld, l. c., p. 39.

LESSON XXIV.

REPRODUCTION OF THE FUNGI AND LICHENS.

IN the months of May and June one may frequently find on the underside of the leaves of the barberry, *Berberis vulgaris*, orange-colored warts which appear to the naked eye to be finely punctured. A magnifying glass will show that the pillow-like swellings are surmounted by minute orange-red cups. The corresponding place on the upper side of the leaf is marked by a reddish fleck bordered with yellow. The magnifying glass shows it to contain in the inner parts numerous brown points bordered with orange-red, similar points being found on the edges of the swelling on the underside of the leaf. The little cups are the æcidium fruit of *Æcidium berberidis*, the spermagonia of which are the above-mentioned dark points. Both together form the first generation of our common rust-fungus, belonging to the *Æcidiumycetæ* or *Uridineæ*, *Puccinia graminis*, which completes its second generation on our corn and other *Gramineæ*, producing there the rust disease (1). Prepare a delicate section of the leaf through the swelling and examine it with first lower and then higher powers. We assume that the material is fresh, though good alcohol material will answer. Treating with potash lye will satisfactorily clarify the fresh section. The cell-layers of the healthy part of the barberry leaf are as follows—the upper epidermis, a single layer of elongated palisade parenchyma, a layer of loose sponge-parenchyma about five cells high, the lower epidermis. The tissue of the affected place is about twice the thickness of the leaf. Upon the palisade cells which are higher but otherwise

little changed, is joined a close tissue more or less elongated in a direction perpendicular to the surface of the leaf, and is distinguished from the adjoining sponge parenchyma by its lack of intercellular spaces. The epidermis of neither surface has been changed. The cell contents of all these cells have undergone disorganization, and consist in part of colorless drops of oil, in part of greenish-yellow and reddish drops arising from the chlorophyll grains and cell-plasma, and of granular masses. The whole tissue of the affected part shows its intercellular spaces penetrated by delicate hyphæ, occasionally branched, articulated by division walls and containing drops of oil. They extend to the epidermis on both sides. With chloriodide of zinc and also with iodine and sulphuric acid, the blue color is not induced in them, as fungus-cellulose rarely shows that reaction. Our section of the little cups shows them to be more than half embedded in the tissue of the swelling. We may easily see that the mycelium forms a thick layer under the little cups out of which arise numberless club-shaped hyphæ, perpendicular to the layer and parallel to each other, solidly packed together and forming the so-called hymenium. These hyphæ, the basidia, are transformed at their ends into a straight series of spores, which though in the basidia colorless, and by mutual pressure polygonal, gradually round out and become orange-red. The spores separate from each other higher up and are discharged from the opened fruit vessel. An examination of the youngest spores on the basidia teaches us beyond doubt, that they are successively separated from the point of the growing basidia by means of a transverse wall. The single layer which constitutes the wall of the peridium, or fruit-cup, consists of cells which look like spores but which remain polygonal and adhere together laterally. Their fine, delicate, porous walls are much thickened on the

outside. The growing peridium presses through the surrounding tissue of the leaf, tears open the epidermis, and so comes forth. The pear-shaped spermatogonia, mainly embedded in the upper side of the leaf are like the æcidia-spores, surrounded by a thick plexus of hyphæ, from which spring closely compressed parallel threads which run toward the middle of the organ. These filaments are very slender, and those found on the upper part form a delicate bundle which protrudes from the organ. These threads are called the sterigma, are transformed at their tips without into small globular cells, the spermatia, which are discharged from the organ as a shiny mass. The sterigma themselves bear orange-red oil drops, which lend their color to the whole body of the organ, particularly to the outside. The spermatia do not germinate. Their significance is unknown. One might be inclined to consider them the product of the male organ and to suppose that a generative act introduced the formation of the æcidium fruit. As already mentioned, this fungus lives in a second generation on the *Gramineæ*. It belongs to the "heterecious" parasites, which in opposition to the "autoecious" complete their circuit of life on different hosts. The proof of this is obtained by sowing the æcidia spores on the germinating plants of cereals (2).

The uredo growth of *Puccinia graminis* meets us only too often in nature from the middle of June till fall, on rye, wheat, barley, oats, and particularly on couch-grass, *Triticum repens*. It attacks principally the stem and leaf sheath of the infected plant. One recognizes it easily as the slender, rusty-brown colored stripes, parallel to the nerves of the leaf, several centimeters long. The epidermis of the host will be seen torn and lifted up by the underlying layer of spores. First appears the rust-colored layer of uredo spores, with which are gradually associated the brown teleutospores. Gradually the uredo spores are

changed, at last fully, till the layer becomes dark, almost black, and towards the end of summer only teleutospores are to be found. If fresh material is not at hand, alcohol material, even dry plants, will serve for examination. Make a transection of the stem of an infected plant. We may easily demonstrate that the hyphæ permeate only a definite tissue of the part. It is the loose chlorophyll-containing tissue stripe, which alternates with sclerenchymatous thickened stripe in the stem, and which is covered with an epidermis that is provided with stomata. Here the cells are thickly interwoven with the jointed hyphæ and their contents disorganized. At those points where the section cuts a layer of spores one sees the mycelium with many short and delicate branches spring up towards the surface, which are headed off at their swollen ends into a unicellular spore, the uredospore. The epidermis is cracked open and its edges laterally raised up. The spores are in different stages of development. The ripened ones are a longish oval and with a sufficiently strong magnification two layers may be seen in the envelope. The outer dark brown is beset with numerous small warts; the inner and less dark shows several, mostly four, pits, regularly divided at the equator. The contents of the spore are granular and the inner portion a lively orange-red.

A transection through a stalk of oats, having the dark brown teleutospores, shows us that the cause of the hyphæ is the same as previously seen. The teleutospores are borne on a somewhat thicker-walled style than the uredospores. They are bicellular oval with the two large ends turned together. The envelope is dark brown. The plants investigated in the course of the season will show both kinds of spores.

The teleutospores survive the winter and are capable of growth the next spring. Each of the two cells puts out

a delicate tube, the so-called promycelium which divides transversely into several cells and from these issue variously shaped processes which divide at their ends into kidney-shaped sporidia. These will infect the barberry leaves; if they fall upon one sufficiently young, the germinating tube penetrates the outer wall of the epidermal cells directly into the inside of the leaf of the host. We therefore see that the infecting of the leaf does not altogether depend upon the germinating tube entering a stoma.

In order to become acquainted with the structure of the hymenium of the *Hymenomyceles* (3), we will select one of the numerous species of toadstools (*Amanita*), mushrooms (*Psalliota*), or agarics (*Russula*). We will take a *Russula* because it possesses one of the already mentioned cystides. Upon the underside of the cap are the radial lamella which bear the hymenia. Cut a piece out of the cap parallel to the course of the lamella and make the thinnest possible transection of the whole perpendicular to the latter. The whole section will resemble a comb, the sections of the lamella forming the teeth. With a low magnification, we shall see that the hyphæ go down from the cap-disk into the middle of the lamella, thence by repeated lateral branching extend to the sides of the latter. A portion of these branches swell into club-shaped forms and end blindly, but a greater part of them remain slender and form outside of the club-shaped branches a compact layer of tissue, of roundish articulations, which is known as the sub-hymeneal layer, and is more or less sharply differentiated from the inner tissue mass of the lamella, the so-called "trama," or woof. The club-shaped branches of the trama serve to give the needed stiffness to the lamella. The basidia and the paraphyses spring from the sub-hymeneal tissue, Fig. 89. They are nearly parallel with each other and set perpendicular to the sides

of the lamella, forming the hymenium. The basidia, *b*, are club-shaped. At their flattened ends are formed four slender branches, *c*, the sterigma, which swell out at their ends into an ellipsoidal cell, the basidia spore, *sp*. These spores, after attaining their full size, remain smooth in most cases, but in many *Russula* species, have short spines on their surface. See Fig. 89. They are separated from the sterigma by a division wall and finally fall off. The spore carries with it a small portion of the sterigma. The paraphyses, *p*, are smaller sterile basidia. So far the toad-

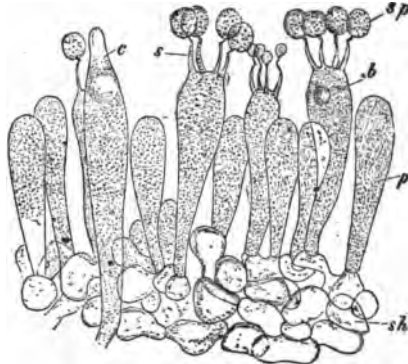


FIG. 89. *Russula rubra*, a portion from the hymenium. *sh*, sub-hymenial layer; *b*, basidia; *s*, sterigma; *sp*, spores; *p*, paraphyse; *c*, a cystid. $\times 540$.

stoals and mushrooms agree with the description of the agarics. But in the agarics occur a few cystides, *c*, between the basidia and the paraphyses, which are as stout as the basidia; their pointed ends protrude beyond the general surface of the hymenium, and with their slender base penetrating the sub-hymenial layer, they represent as direct branches, the median elements of the trama. All these elements named above are separated at their base from the hyphæ by division walls, contain finely granular plasma, and often single drops of oil.

In order to become acquainted with the highly developed form of the hymenium of the *Ascomycetæ* we will select for examination, *Morchella esculenta*. Dried specimens may be soaked out and used, but fresh plants are naturally to be preferred. This well-known morel has an irregularly egg-shaped, stalked fruit-body, which conceals a simple cavity within, and whose upper expanded part is laid in deep folds. The sunken portions are lined with hymeneal tissue, which has not been developed in the projecting ribs between. Make a section perpendicular to the surface of some one of the depressions. The hymenium consists of spore sacs laid almost parallel with each other, (asci) sap filaments (paraphyses), Fig. 90. The spore-tubes, *a*, are nearly cylindrical and contain in their upper part eight ellipsoidal single-celled spores, closely pressed together. The ascus also contains a highly refractive epiplasm. The paraphyses are brownish filaments, articulated with division walls and slightly smaller at the top. The upper cell is the longest. The filaments are not as long as those of the asci. Both elements are the ends of the hyphæ of the closely-interwoven superficially-extended, sub-hymeneal tissue. This rests on the loosely-built hyphæ tissue of the fruit body. Treating the section with potassic iodide of iodine colors the epiplasm of the asci reddish-brown. This is a characteristic reaction for epiplasm and has recently been designated the glycogen reaction (4). A characteristic peculiarity of this reaction



FIG. 90. A part of the hymenium of *Morchella esculenta*. *a*, asci; *p*, paraphyse; *sh*, sub-hymeneal tissue. $\times 240$.

shows itself by the application of heat. To a section in water, stained with the iodine reagent, add a little more water but not enough to remove the color. Then gradually and carefully warm it without bringing it to the boiling point, laying it over white paper occasionally to see if the color becomes paler. When this takes place, rapidly cool the preparation, and if it is a large one it will be seen by the naked eye to take on its dark color again (5). By means of potassic iodide of iodine, one may trace the beginnings of the asci from some depth in the tissue of the sub-hymeneal layer. The paraphyses, the sub-hymeneal layer, and the tissue of the inside of the fruit-body are colored at the same time a yellow, or yellow-brown color.

The fungus in the thallus of the lichen belongs, with rare exceptions, to the *Ascomyceteæ*. The *Physcia ciliaris* is rich in fruit. The apothecium is saucer-shaped with an inclosing border formed from the thallus. This diminishes under the apothecium into a pedicel a transection of which shows a radial structure, with a uniform thickness of rind layer following which is a layer of gonidia around the whole circumference. The inside of the pedicel is occupied by a loose texture of hyphæ.

We make next a median longitudinal section through the apothecium. This shows the structure of the border of the apothecium constructed out of the tissue of the thallus. The gonidia layer extends to the edge of this border, from which at intervals cilia-like processes put out. The style widens to inclose the hymenium, which rests on its central fundamental tissue. The hymenium is brownish. It consists of a great number of long, extremely slender, jointed filaments, the paraphyses, between which, far less numerous, stand the club-shaped spore-sacs, the asci. The latter are always of different age, the ripe ones having eight brown-walled spores. The

spores are ellipsoidal, bicellular and at the boundary of the two cells a little contracted. Both elements spring from a felted, uniformly colored, horizontally extended layer, the sub-hymeneal layer. This rests upon the central tissue of the style from which it is distinguished by its brown color and its lack of air-filled spaces. While we have seen that the hyphæ of the thallus are not colored blue with chloriodide of zinc, the hymeneal tissue is colored a dark-blue by the application of a little potassic iodide of iodine. The walls of the hymeneal elements are formed out of a particular modification of cellulose, which is known as starch cellulose. Examining the thallus of this lichen with a magnifying glass we shall find little wart-like elevations standing here and there singly or in groups. If we make delicate transections in considerable number through the thallus we

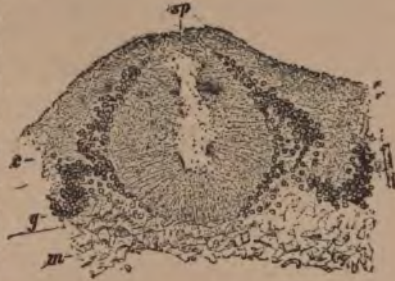


FIG. 91. Transection of thallus of *Physcia ciliaris* through the middle of a spermatogonium, *sp*; *e*, rind layer; *m*, pith; *g*, gonidia layer of the thallus. $\times 90$.

shall, with some of them, hit one of these elevations in such a way as to show a section like that represented in Fig. 91. This is the spermatogonium, an egg-shaped form, sunk in the thallus and having an open pore or mouth, *sp*. It occupies nearly the whole depth of the thallus, is surrounded on the sides by the gonidia layer, and has within a mass of very delicate, nearly radially-arranged filaments with short joints, the sterigma (see the figure). The longer axis of the organ is occupied with a cylindrical cavity which contains short rod-like spermatia which have

been separated from the ends of the sterigma. These escape through the opening at the top of the spermatogonium. In the *Collema* it has been demonstrated that the function of the spermatia is that of the male generative product (6). In other lichens their function is still unknown.

NOTES.

(1) See de Bary, Monatsber. d. k. Akad. d. Wiss. in Berlin für das Jahr 1865, p. 15; Kny, Bot. Wandtafeln, p. 68; Frank, die Krankheit d. Pflanz., p. 454.

(2) de Bary, same work, 1866, p. 206.

(3) See de Bary, Morph. u. Phys. der Pilze, p. 112; Goebel, Grundzüge, p. 143. In both the rest of the literature.

(4) Leo Errera, L'épithème des Ascomycètes, 1882. There also the literature relating to epiplasma.

(5) l. c., p. 45.

(6) E. Stahl, Beiträge zur Entwicklungsgeschichte der Flechten, Heft 1, 1877.

LESSON XXV.

REPRODUCTION OF THE MOSSES.

THE *Marchantia polymorpha*, already known to us, is most rapidly propagated in an asexual or vegetative way by means of asexual buds or gemmæ. They are common in the *Hepaticæ* generally and appear in most exquisite form in this species. They are produced in the *Marchantia* in cup-shaped receptacles on the back side of the thallus. The cup has a beautifully toothed border and the vivid green gemmæ are found at the bottom. A longitudinal section through the cup, parallel with the long axis of the thallus, first narrows and then pretty suddenly widens outward to the edge. The tissue forming the air-chambers continues up the outside of the cup to the upper half of its outer extension. The base of the cup is occupied with club-shaped papillæ whose membrane is transformed into mucilage. Between these club-shaped hairs are occasional bicellular hairs whose upper cells are divided first by transverse walls and subsequently by longitudinal walls till they at last attain a considerable lateral extension, and finally become several cell-layers thick in the middle and quite biscuit-shaped in form (1). The single-celled styles are easily parted leaving the gemmæ loose in the cup, from which they are soon discharged by means of the swelling mucilage which is produced in the bottom of the cup by the club-shaped hairs. The little notches on the side of the gemmæ form the vegetative points whence are produced short papillæ. The cells of the gemmæ are rich in chlorophyll; still on both surfaces of the organ are found large chlorophyll-free cells, which keep near the middle

but are otherwise irregularly distributed. In some of the border cells are oil-bodies. When the gemmæ are sown and germinate, these chlorophyll-free cells develop in a day or two on the under side into root-hairs, and on the upper side into the tissue of that side (2).

The sexual reproductive organs of *Marchantia* are placed on special receptacles. We will examine those of *M. polymorpha* (3). The male and female receptacles are easily distinguished, the former presenting disk-like and the latter umbrella-like forms. The two organs are produced

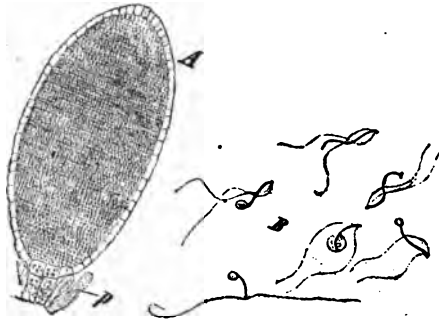


FIG. 92. *Marchantia polymorpha*. A, optical transection of a nearly ripe antheridium; p, paraphyse; B, spermatozooids fixed with a 1% solution of perosmic acid. A \times 90; B \times 600.

on different plants. The receptacles together with their styles represent the transformed branching of the plants. By making a delicate section through the pistillate receptacles, we see that its structure conforms to that of the thallus, its upper surface answering to that of the back side of the frond and the under side of the receptacle to the under or ventral side of the frond, being provided like that with rhizoids and scales. The antheridia, Fig. 92, A, are sunk in special cavities in the open side of the male organ. The section shows that each cavity contains but

one antheridium together with a few short single-celled paraphyses, *p*. The cavity closes over the antheridium with the exception of a narrow canal which is left open. The antheridium is an oval body with a short pedicel and has an outer membrane of a single layer of cells containing chlorophyll. The special mother-cells of the spermatozoids are produced by successive right-angular cell divisions, and form a series of transverse and longitudinal rows in the nearly ripened antheridium (see the figure). Just before the ripening of the mother-cells of the spermatozoids they are rounded out and separate and finally burst the enclosing membrane of the antheridium at its apex, and the small round cells escape. If we put a drop of water on the top of one of these growing receptacles, we shall see it spread rapidly over the whole surface and soon become milky-white. A high magnification shows it to be filled with numberless spermatozoid cells. They remain for a short time at rest after which the cell membrane begins to swell up and finally bursts and the spermatozoids escape into the water. The spermatozoids are relatively very small, have a filiform body and two long cilia, and attached to their posterior end a minute bladder which they finally lose during their swarming. In order to see them distinctly, we may add to the preparation a drop of a one per cent solution of perosmic acid which will fix them most beautifully and allow us to study them very conveniently. See Fig. 92, *B*. The same result is obtained by the use of a trace of potassic iodide of iodine.

The female receptacle forms, as does the male, a radially arranged inflorescence generally consisting of nine rays between which are eight rows of archegonia on the under side. This distinguishes it from the male organ. Still this difference depends upon the earlier deflection of the

vegetative point of the receptacle towards the underside. We shall see by the use of the magnifying glass that the row of archegonia lying between the rays is inclosed by a common, one-layered, rib-like membrane, bordered at the edge. By making a delicate, longitudinal section between thumb and finger of a relatively young receptacle,

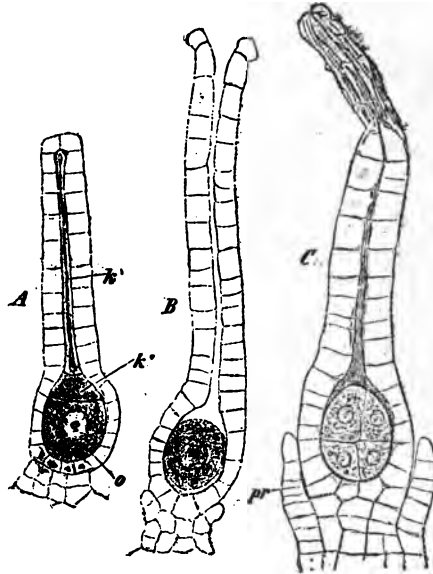


FIG. 93. *Marchantia polymorpha*. *A*, young, *B*, open archegonium, after the formation of the beginning of the germ; *k*, neck canal cells; *k'*, ventral canal cells; *o*, ovum; *pr*, perianthium. $\times 540$.

we shall easily find in some of them the female organ, the archegonium. The oldest lie near the border, the successively younger nearer the pedicel. The first, the ripened ones, show their necks bent upwards; the others run straight downwards. In an archegonium, which is nearly ripe, Fig. 93, *A*, a short pedicel, a ventral and a neck part, may be distin-

guished. The wall of the pedicel and the ventral part is composed of one layer of cells. The central cell of the ventral part is filled with an egg, o , and a ventral canal cell, $k' k''$, which shortly before ripening is separated from the egg. The nucleus of the egg is easily seen. The neck has a central canal running through it which is formed by four canal-cells whose division walls have been absorbed. The contents of these four cells have commingled and formed a continuous string. Between the archegonia are small leaf-like scales originating in the receptacle. In many preparations one will find the membrane which covers and protects the whole archegonium layer. It consists of a single stratum of cells, is fringed at the border, and its cell often contains oily bodies. It is relatively easy to see the opening of the archegonium directly under the microscope.

Make a longitudinal section of the female flower which is raised but a little from the pedicel and lay it dry under a cover glass upon the microscope. When a ripe archegonium is found, add a drop of water at the edge of the cover-glass, keeping the preparation under observation, whereupon the archegonium will almost immediately open. The cause of this opening is in the swelling of the contents of the canal in the neck. The canal cells of the neck dissolve at the apex and their contents escape followed by that of the ventral canal cell. The homogeneous part of these contents forms a rapidly swelling mucilage which is distributed in the surrounding water. The granular contents lie in the water and gradually disorganize. Directly after the emptying of the ventral canal cell, the central cell of the ventral part is rounded up. See Fig. 93, *B*. On its anterior border, is often but not always found a clear spot, a germinal fleck or embryo sac. The introduction of the spermatozoids into the canal of the neck may

be easily observed in this plant. For this purpose instead of pure water, we must add to the preparation a drop of water which has been for a time in contact with a ripe male receptacle. The spermatozoids collect about and in the mucilage which has exuded from the archegonium, and one sees them enter the neck where they become invisible. A substance is secreted from the archegonium which affects the spermatozoid as a chemical irritant and determines the direction of its movement; so, when it reaches the exuded mucilage, it is gradually moved along in the direction of the opening of the neck of the archegonium. It is interesting to notice that the neck of an unfertilized archegonium does not close up, and that the archegonium in such a condition gradually perishes. But if water containing spermatozoa be added to the preparation and the egg be fertilized, the neck is shut up in a few hours by the gradual narrowing of it from above downward. If the preparation be laid by for twenty-four hours, the existence of a cellulose membrane about the fertilized egg may be easily recognized, which gradually thickens in the next following days.

The fertilized archegonium, which one finds in a section, shows a brown shrunken neck, while the egg is already undergoing segmentation, Fig. 93, *C*. About the base of the archegonium there begins to develop from the foot of the same, a cup-shaped envelope, the perianth, *pr*, which incloses the whole growing archegonium. In a longitudinal section of the receptacle, which has already expanded its marginal rays, one may see fixed the living-green, fully-developed archegonia, with their broad bases, and their apex adorned with what remains of the neck part. From the fertilized egg, or ovule, is produced the sporogonium which one sees, finally, in preparations made from the older receptacles. These sporogonia form a yellow-green

oval capsule with a short pedicel. The walls of the capsule consist of one layer of cells. By tearing the membrane apart with needles and examining with a higher power, we observe a characteristic thickening ring in the otherwise thin-walled cells. The yellow-brown spores are finely dotted. Between them are long slender cells, pointed at the ends and characterized by two brown, screw-shaped bands in their walls. They are the so-called "elaters." The interior of the capsules are filled exclusively with spores and elaters. The open capsule has the opening set round with several recurved teeth. The elaters are strongly hygroscopic, bend back and forth by a change in the humidity of the atmosphere and so help to scatter the spores. All *Marchantia* do not have their reproductive organs upon such elaborately constructed receptacles, and in other *Hepaticæ* these specializations generally are wanting. On the contrary, it often happens that the pedicel of the sporogonium is considerably elongated, and the capsule with the spores correspondingly elevated, which also promotes the scattering of the spores.

For a study of the antheridia of the true mosses, *Musci*, we will choose *Mnium hornum*, a widely distributed plant, which forms remarkably fine and numerous male flowers in the month of May, and at the same time offers female blooms, or archegonia, for investigation. The former are much more numerous than the latter, the latter having sometimes to be sought for a long time. The male blooms are dark green, disk-shaped, inclosed in a rosette of foliage leaves, the so-called envelope or perigoneal leaves. Towards the middle, the leaves of the bloom rapidly diminish in size. In the axils of the outer, but also still more in that of the inner leaves, are found numerous antheridia and paraphyses which extend over the whole apex of the stem. This may be easily seen in a longitudinal

section of the bloom, the section being made between the fingers, and the apex of the stem being turned downward in making it. This section shows that the stem is widened at the top and a little hollowed out also where the reproductive organs are inserted. The central conducting bundle, peculiar to the *Mnium* species, is also correspondingly widened and ends in a chlorophyll-containing tissue which is spread out under the bottom of the blossom. The antheridia and the paraphyses are easily made out and their structure ascertained. The antheridia are club-shaped bodies, somewhat contracted at the ends and borne on short pedicels. The cells of their one-layered walls contain numerous chlorophyll grains. The contents consist of small colorless cells whose division walls in the young state of the antheridium are clearly placed at right angles. If older antheridia are cut by the section, the exuding contents are seen to consist of rounded, adhering cells, the spermatozoid cells in which the filamentous bodies of the spermatozoids may often be recognized. The chlorophyll grains at the apex of the ripe antheridia are brownish. Empty antheridia are opened at their apex. The paraphyses are simple cell filaments which gradually expand upward but again contract so that the uppermost cell is always sharp. The walls of the cells are often brown at the base of the paraphyses and sometimes higher up also. They bear chlorophyll. Cross-sections made through the under part of the bloom show in an instructive way the distribution of the antheridia, their relations to the enveloping leaves and to the paraphyses, also many transections of the antheridia themselves.

Still more satisfactory than the male blooms of the *Mnium* are the red-colored ones of the *Polytrichum* species which may be likewise found in the month of May. Select *Polytrichum juniperinum*. The perigoneal leaves dif-

fer from the foliage leaves not only in color but also in the fact that the single-layered sheath-part extends quite to the apex of the leaf. The formation of the green lamellæ, characteristic of the *Polytrichum*, is limited to the nerve on the upper side of the leaf. On the red-brown perigoneal leaves which occupy the inside of the bloom and become rapidly smaller, are the green lamellæ produced, and only on the outermost leaves at the point which is bent sharply outward. So the leaf appears finally to be reduced almost to its sheath part. The antheridia and the paraphyses stand in the axils of the perigoneal leaves. The middle of the bloom is occupied with a vegetative bud which is a continuation of the central string of the stem. In *Polytrichum normale*, the male flower grows up through this. The antheridia have the same structure as in *Mnium*. The paraphyses form a long cell-filament at their under part, widen spatulate at top into a single cell layer. If one presses a bloom of the *Polytrichum* between the fingers, the contents of the antheridia will exude as a milky slime clearly visible on the red-brown leaves.

The female blooms of *Mnium hornum* are generally not so easily seen as the male and one must hunt for them. The plant which bears them is much shorter and has darker leaves. The upper leaves close bud-like about the pistillate organ, the archegonium, to protect it. The median longitudinal section shows that the stem is not essentially widened at the top but is blunted in a peculiar way. This may be a sure indication to us that we have to do with a female bloom even when we cannot find the archegonia. The central conducting-bundle of the stem is somewhat enlarged under the bottom of the bloom and ends, as in the male flower, in a tissue containing chlorophyll. The periechætal leaves while remaining foliaceous in form diminish in size towards the middle of the bloom. In hermaphro-

dite flowers they form what is called the perigonium. There are but few archegonia in the apex of the flower so there must be an exactly median section in order to find them. The archegonia are in the main formed on the same plan as those of the *Hepaticeæ*, only that the pedicel is much more strongly developed and but little diminished below, forming the principal mass on the under half of the archegonium. The ovum appears relatively small on this foundation. It must be sought for immediately under the beginning of the neck which is here but a little more slender than the ventral part. The chlorophyll contents of the cells make the archegonium less transparent, hence for the most part the ovum and the canal cells of the neck will be visible only after the section has been treated with potash. In the axils of the perichaetal leaves are many short paraphyses. They consist of a series of short cells somewhat smaller towards the top, the lower ones often being brown.

We will now undertake the study of the sporogonium of this plant, *Mnium hornum*. It is the so-called "fruit" of the moss. It consists of the capsule and the pedicel or style. The bottom of the latter is embedded in the tissue of the mother plant. The "hood" (calyptra) which arises from the enlarged archegonium, and which covers the young capsule, will be cast off very early and will therefore be difficult to find. It is composed of one and in part also of two layers of elongated cells and is split on one side quite up to its slender apex. The apex ends in a brown point which corresponds to the neck of the archegonium. At the bottom where it was broken by the growing sporogonium it appears as if cut off. The top of the capsule from which the calyptra has been removed is covered with a lid which is provided with a short beak. It may easily be removed by means of a needle

and then the edge of the urn-like capsule with its teeth come into view. The teeth constitute the peristome. The upper part of the style, which is transformed into the capsule, is called the apophysis. In the present case the latter is separated from the capsule by a slight contraction and is distinguished by its brown color. In some of the foliaceous mosses the apophysis is much stouter than the capsule, as in the *Splaehnceæ*. To study the structure of the peristome, we should cut a transection of the capsule immediately under the edge of the urn, and transfer it to the slide with the teeth upward. Adjust the mirror and view the object with reflected light using a low magnification. We observe that the teeth are inserted on the inner edge of the urn, are pointed, wedge-shaped and striated across. If we breathe lightly upon the object during the observation we find that the teeth bend together inward. They are hygroscopic and bending inward in moist weather close up the open capsule, while in dry weather they bend back outward and again open the capsule. There are sixteen teeth upon the edge of the urn. Put such a section, which has been cut open on one side, in a drop of water, laying it flat down, and place a cover-glass over it; examining it with transmitted light, first from its outside, we see that there is a double layer of cells, about the edge of the urn, which is composed of inclined, thickened, papillaceous, chlorophyll-bearing cells with colorless walls, browned only at the base, where they are easily detached all together from the brown edge of the urn. These cells form the so-called "ring" at the edge of the capsule and mark the place where the cover separates. By turning the inside of the preparation upwards, we see that the cross-markings on the teeth are caused by projecting ledges on the inside. Inside of these teeth are the so-called "cilia." Consequently, this plant has a

double mouth-piece, while the *Bryaceæ* possess but one. The teeth and the cilia are flat lamellæ which appear to be divided off below into compartments, and to be cross-stripped above with low projecting ledges on the inner surface. Below, it is united into a continuous membrane which is arched a little between each two teeth of the outer mouth parts. Each two cilia stand between two teeth and present themselves obliquely from the edge. Their edges are beset with serrate projections, the outer along their whole height, the inner only on their upper parts. The transverse ledges or projections of the free parts of the cilia end in these teeth. By means of these teeth the edges of the cilia are united together in their upper part and finally form a long slender point. Alternating with these pairs of hairs are from three to five very slender ones which stand opposite the outer teeth.

A delicate transection made somewhat deeper through the capsule shows within, the so-called column formed of large-celled tissue. About this column lie the spore-filled spaces. The inner walls of these are formed by the column itself, the outer by a double layer of tissue containing chlorophyll, which is separated from the walls of the capsule by a loose tissue of cells also containing chlorophyll. The walls of the capsule consist of two to three layers of cells and are covered by a distinctly marked epidermis, the cell-walls of which are much thickened on the outside. The spores contain chlorophyll grains, their walls are brownish, beset with warty protuberances, and in favorable cases a three-sided pyramidal outline is presented, which is caused by the mother-cell dividing into four spores, and these flattened pyramidal surfaces are where the spores come in contact and press upon each other in the mother cell.

An exact median longitudinal section, made through a

green full grown capsule, on which the cover still remains, will show that the latter consists of a layer of brown much thickened cells without, and several layers of thin-walled cells within. On the boundary between cover and capsule lies the double layer of obliquely-placed chlorophyll-containing cells, already known to us, on which the separation of the lid from the capsule depends. The next cells of the capsule walls below are very short. Adjoining these small cells on the inside are thickened brown-colored cells, which form an inwardly projecting ledge on which are set the outer teeth of the capsule. About one cell thickness beyond arise the cilia. The history of the development of these teeth and cilia shows them to have been produced by local thickening of the opposite walls of one and the same cell layer on the inside of the cover. The teeth arise from definite portions of the outer wall which are connected together in an ascending direction, whose transverse ledges correspond to inner adjoining transverse-walls on which the thickening has continued a certain distance beyond. The cilia arise from the thickened parts of the inner walls of this cell layer and bear low ledges on the places nearest the attachment of the inner division walls.

In our median longitudinal section the cover is hollow. After the completion of the teeth and cilia, the inner tissue shrivels up and separates from the inner surface of the cilia which reach to the apex of the cover. This tissue forms a cone-shaped, projecting knob on the top of the column. The latter is visible along its whole length. We see also the spore-sac, its outer wall, the loose tissue which lies between it and the wall of the capsule, finally the latter also. The spore-sac is closed, while the cover remains intact by a thin layer of tissue, which is after-

wards ruptured. At the bottom of the capsule, under the spore-sac, a ring-shaped cavity is formed. The apophysis is provided with stomata, almost every median longitudinal section touching one of them. They lie under the epidermis. A canal leads down and a breathing cavity is at the end of it. They are surrounded by chlorophyll-containing tissue, whose intercellular spaces communicate with the ring-cavity at the base of the spore-sac, and also with the air cavities in all the chlorophyll-containing tissue which lies between the spore-sac and the wall of the capsule. All the stomata are here viewed longitudinally and agree in form with those of the vascular cryptogams and phanerogams. The apophysis, and in other cases the wall of the capsule also, furnish the only places in the mosses where genuine stomata answering to those of the higher plants may be found.

To complete our view of the structure of the vessel of the moss-fruit, we will make a superficial section of the capsule and the apophysis. We demonstrate the want of stomata in the surface of the capsule. Between the brown-walled cells of the apophysis we see the canals which lead down to the stomata. Turning the section over and examining it from the inside, we shall find, in favorable cases, the guard-cells of the stomata as in the higher plants. We shall also observe that the green cells lying between the spore-sac and the walls of the capsule are connected together in the direction of their length, that they are branched, and altogether appear quite like filaments of algæ. A transection through the apophysis mostly touches those stomata whose two guard-cells are easily seen. The epidermis proper ceases, the surface being occupied by two or three layers of yellow- to red-brown, much thickened cells, whose inner cavity gradually increases in size

towards the interior of the stem. In the inside of the stem a conducting bundle is differentiated. A median longitudinal section in the neighborhood of the apophysis shows that these relations when begun in the stem very gradually develop themselves.

NOTES.

(1) Goebel, die Muscineen in Schenk's Handbuch der Botanik, Bd. II, p. 338.

(2) See A. Zimmermann, Ueber die Einwirkung des Lichtes auf den Marchantienthallus, Arb. aus d. bot. Inst. in Würzburg, Bd. II, p. 665.

(3) Leitgeb, Untersuchungen über die Lebermoose, VI Heft, 1881, pp. 20, 117; Goebel, *l. c.*; Strasburger, Jahrb. f. wiss. Bot. VII, p. 409, und Befruchtung und Zelltheilung, 1877, p. 12.

LESSON XXVI.

THE REPRODUCTION OF THE VASCULAR CRYPTOGAMS.

WITH rare exceptions the sporangia of the ferns are found on the under side of the leaf, mostly forming groups, called sori. Often the whole sorus is covered over by a growth from the leaf called the indusium. The indusium is very differently developed in different cases. Sometimes the edge of the leaf folds over the sorus forming what we call a false indusium.

Take for investigation the *Scolopendrium vulgare*. A prominent mid-rib runs through the leaf from which branch out laterally smaller nerves a little inclined forwards. The sori are developed on the upper half of the fertile fronds, lying in the same general direction as the lateral nerves. From without they appear to be covered more or less perfectly with two lip-like indusia which at first overlap each other and afterwards gape open. Make a transection of a piece of the fertile frond choosing one whose sori have become brown, but whose indusia have not yet begun to gape. Cut out a piece of the frond with the shears, parallel to the sori, and make a delicate section by means of elder-pith. The transection, Fig. 94, *A*, shows us the epidermis of the upper and under side, and the sponge-parenchyma which closely joins the former. The apparently simple sorus-stripe is found to be double, the parts inclined towards each other right and left, and each close upon a vascular bundle. The surface of the leaf is here deeply channelled, with a projecting edge between the two sori. The epidermis at the bottom of the channel, on which the sporangia are growing, lies im-

mediately upon the sheath of the vascular bundle. The epidermis of the under surface of the leaf and that of the canal unite to form the indusia, *i, i*. These begin in a double layer of cells, but soon pass into a single layer which has the structure of the neighboring epidermis, ex-

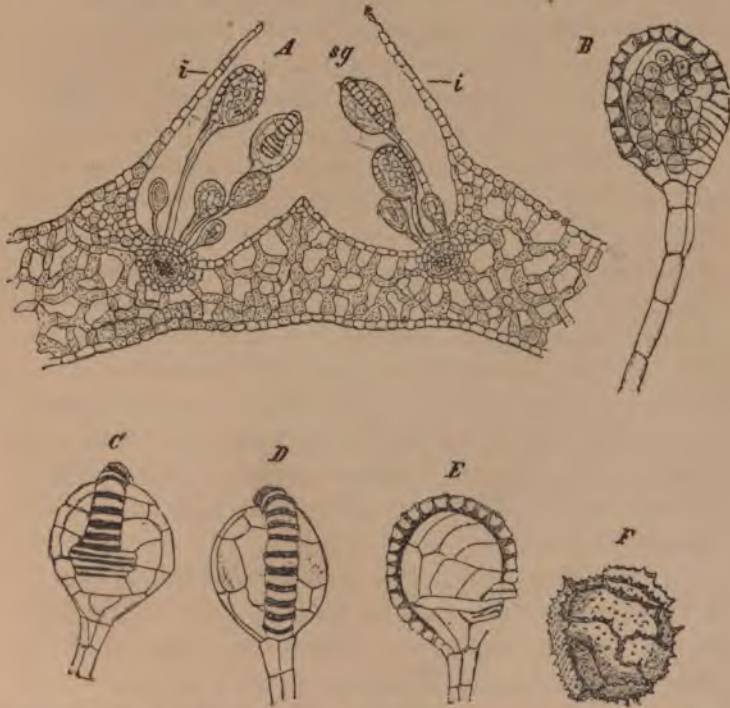


FIG. 94. *Scolopendrium vulgare*. *A*, transection through the fertile leaf; *i*, indusium; *sg*, sporangium; *B-E*, sporangia: *B* and *E*, seen from the side, *D*, from the back and *C*, from the front; *F*, a spore. *A* $\times 50$; *B-E* $\times 145$; *F* $\times 540$.

cept in lacking stomata and chlorophyll grains, although colorless chromatophores are found in it. At the bottom of the channel we see the sporangia, *sg*, in different stages of development. Each arises from an epidermal cell. By the use of a low magnification, Fig. 94, *A*, we distin-

guish in each sporangium, a style, a capsule, and in the older ones a yellowish-brown ring on the capsule. With a higher magnification we find that the style passes from a single into a double series of cells and the walls of the capsule consist of a single layer of cells, Fig. 94, *B*. As the different views of the wall of the capsule show, *B-E*, the ring is formed of a series of cells in the wall which project outwardly, beginning at the style, running over the top of the capsule to the opposite side where they broaden and flatten and finally end without reaching the style again. The inner and transverse walls of the ring-cells are much thickened and browned, the thickening on the transverse walls diminishing outwardly. The sporangium opens between the broad cells in which the ring ends, Fig. 94, *CE*, half of the broad cells coming on one side of the transverse cleft and the others on the other side. The origin of the springing action of the sporangium lies in the ring which by drying produces an outward tension. The brown walls of the ripe spores show a beautiful structure, Fig. 94, *F*. The outer surface is covered with comb-like projecting ledges united together in a netlike form.

In *Aspidium felix-mas* we find the heart-kidney-shaped indusium which in age is lead-colored and at last becomes brown and somewhat shrunken so as not perfectly to cover the dark brown sorus. The sporangia have the same structure as in the *Scolopendrium*. On the style of some, a short glandular hair is found. The sporangia grow from a cushion-like elevation, the placenta, which lies over a vascular bundle. On the latter are placed reticulated thickened tracheïds which extend into the placenta. At its apex the placenta bears the indusium, inserted with a pedicel-forming sinus. By making a preparation of a ripe but still closed sporangium in water and adding a

dehydrating fluid like glycerine at the edge of the cover-glass, the sporangium will gradually open before our eyes, the ring becoming strongly concave, after which a backward movement takes place by which the sporangium is more or less perfectly closed again. The whole may be repeated with diminished force several times. The sporangium of the *Scolopendrium* shows the closing movement much less perfectly. It will be of interest to examine a naked sorus of the *Polypodium vulgare*. The sori of this genus are entirely without indusia and lie each upon a vascular bundle. The placenta rises scarcely above the surface of the leaf. The sporangia are of the same type as those of the other species.

For a study of the sexual reproductive organs of the vascular cryptogams, and of the process of reproduction, we will also select a fern. The prothallium, the first sexually differentiated generation of the fern, is easily obtained, either by sowing the spores, or collecting the already grown prothallium. For the latter purpose the *Polypodiaceæ*, everywhere occurring and rich in species, will serve us best. For sowing, the spores of the *Ceratopteris thalictroides*, cultivated in every botanic garden, may be chosen. Like that of most other *Polypodiaceæ*, the prothallium of *Polypodium vulgare* has the form of small, heart-shaped, living-green leaflets lying on the substratum. Seize a prothallium of medium size, at the point where it is attached to the substratum and remove it from its fastening. Wash off all the adhering soil in water and lay it on a slide in a drop of water, with the ventral side up, and examine under a cover-glass. The heart-shaped prothallium consists of numerous polygonal chlorophyll-bearing cells. In the anterior sinus lies the small-celled meristem of the vegetative point. The prothallium has several layers of cells only in the middle, the so-called tissue-cushion. It runs out on the sides to a

single layer of cells and gradually flattens itself out towards the base of the prothallium.

The root-hairs or rhizoids spring from the posterior part of the frond, being found mostly in the middle. They are long, single-celled tubes, soon becoming brown. On the under edge of the prothallium single cells develop into short, almost always single-celled papillæ, which, like the rhizoids, are set off at their base by division walls. If we have a young prothallium these are male, if an old one, they are exclusively female reproductive organs. Be-

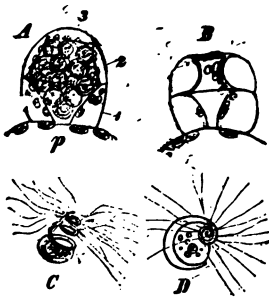


FIG. 95. *Polypodium vulgare*. *A*, a ripe and *B*, an empty antheridium; *p*, prothallium cell; 1 and 2, ring cells; 3, cover cell. *A* and *B* \times 240. *C*, spermatozoid in motion; *D*, one fixed with iodine solution. *C* and *D* \times 540.

tween both stand those which unite the two sexes. The male organs, the antheridia, are found in the posterior part of the prothallium, among the root-hairs and beyond them on each side. They grow at the apex. They appear as spherical arched forms, Fig. 95, *A*, which in the ripe state contain within small globular cells in great numbers. Beyond the ripe antheridia are those already emptied, as their

clearly show. We get a complete view of the structure of the antheridium only when we examine it in profile. This view may be got by bending the prothallium over a needle. This view is seen in Fig. 95, *A*, by which it is observed that the antheridium sets in the middle of a low arched prothallium cell, *p*, from which it is separated by a division wall. The wall of the antheridium consists in almost every case of two stories of lateral cells, 1 and 2, and a cover-cell, 3. The lower has a wider cell cavity than either of the upper ones. The side view of an empty antheridium, Fig. 95, *B*,

shows the lateral cells much swollen and very prominent, the inner cavity of the antheridium consequently much diminished and the cover-cell pressed flat and broken through. If, now, we return to the superficial view of the prothallium and examine the empty antheridium from above, we shall see that the side cells have no division walls, and we perceive that they are really ring-cells. The whole wall of the antheridium therefore consists of two superimposed ring-cells and the cover-cell. Cells of this kind are very rare elsewhere, but constantly reappear in the antheridia of the *Polypodiaceæ*. The only deviation from this form of antheridium among the *Polypodiaceæ* is in the case where the antheridium is borne on a pedicel and consists of but one ring-cell. If we select a prothallium which has not been wet for some little time, we shall not have to wait long for some of the ripening antheridia to discharge their contents. The mechanism for the discharge of the antheridium depends upon the pressure which the ring-cells exert on the cell contents, as well as upon the swelling substance which is secreted among the peculiar contents-cells of the antheridium. The cover-cell will finally be broken, and the contents of the antheridium will be pressed out and the ring-cells will increase in size. The contents consist of isolated, spherical cells, the spermatozoid cells, which coming out into the surrounding water remain at rest for a little while. As may be seen by a comparatively low magnification, a little filament is coiled up in each cell, the spermatozoid, and a central collection of granules may also be recognized. The walls of these cells after a few hours dissolve in the surrounding water and the spermatozoids are set free. This takes place with a sudden movement which uncoils the spermatozoid. One spermatozoid es-

capas after another. We follow these and observe that they move quite rapidly through the water and at the same time rotate about their axes. After about twenty or thirty minutes the motion begins to slacken and soon ceases altogether. During this last stage of the motion of the spermatozoid its form is not difficult to recognize, which may be done all the more easily if we add to the water-drop containing them a ten per cent filtered solution of gum arabic and so diminish the rapidity of their motion (1). The spermatozoid, Fig. 95, *C*, is formed from a band rolled into the form of a corkscrew, the twist being narrow at the front end and growing wider backwards. The forward end bears long, fine cilia. Within the posterior twist lie fine granules and often an inclosing sac may be seen. By the addition of a little potassic iodide of iodine solution the spermatozoids are very beautifully fixed.

At the anterior sinus of the prothallium we shall find the female reproductive organs, the archegonia. Next to the sinus are the unripe ones; beyond, the ripe but unopened, and beyond them still the opened and dead ones brown on the inside. These are very easily distinguished from the male organs. They rise out of the surface of the prothallium, in the form of short cylindrical elevations which bend away from the anterior sinus. These free parts of the archegonia are only the necks, while the ventral parts are sunk in the tissue of the prothallium. The neck is composed of a wall with a single layer of cells in four rows and a central canal whose contents in the ripe archegonium, in the middle, are granular and in the periphery strongly refractive. This inner canal widens club-shaped above. Below it passes into the central cell of the archegonium in which is the ovum. The latter is scarcely distinguishable. If we do not wet the prothallium for



several days before the investigation, we shall probably be able to witness the opening of an archegonium. Take an archegonium, the contents of the canal of which are very strongly refractive. The opening may occur in a moment or we may have to wait a long time for it. The opening of the neck is caused by the pressure of the refractive, expansive substance in the canal on the walls of the neck. The four cells at the apex of the neck suddenly separate and the contents of the canal pour out, distinguishing themselves as a colorless mucilage in the surrounding water, while the granular contents slowly disorganize. The emptying of the archegonium takes place interruptedly, first from the neck canal and then from the ventral canal cell which lies next the ovum.

Under especially favorable circumstances one may see the entrance of the spermatozoids into the archegonium. We shall increase our chances of this if we use an old prothallium having archegonia and a very young one rich with antheridia. Spermatozoids distributed in the water swim quietly by the unopened archegonia; but, if the archegonium has opened, the spermatozoids for a measurable distance round take the direction of the open mouth of the neck, and will be intercepted by the mucilage mass. Within this their motion lessens; still, however, keeping the original direction and follow down the neck to the ovum in which they are absorbed. As has been lately discovered, the neck of the archegonium secretes a substance which exerts a chemical irritation on the spermatozoid which determines the direction of its movement (2). The particular irritating medium in this case is malic acid, about 0.3 % of which enters into the mass which escapes from the neck of the archegonium. The spermatozoids will work their way into capillary tubes in the same manner if they contain a 0.01 % to 0.1 % solution of some base united with malic acid. For the spermatozoids of

the true mosses, cane sugar is the specific irritating medium, while in *Marchantia* another substance, whose nature is not yet ascertained, is produced by the archegonium.

It has been experimentally demonstrated that (3) a single spermatozoid is sufficient to fertilize the ovum. Several, indeed, penetrate into the archegonium, usually, of which only one is really utilized. These processes, however, are not easily followed here on account of the lack of transparency in the tissue of the prothallium. They may be much more easily seen in the *Ceratopteris*. We may, however, demonstrate here that the spermato-

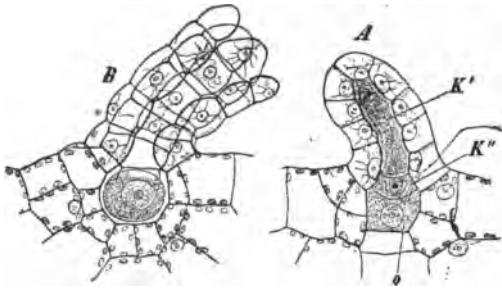


FIG. 96. *Polypodium vulgare*. *A*, unripe archegonium; *K'*, neck canal cell; *K''*, ventral canal cell; *o*, ovum; *B*, ripe open archegonium. $\times 240$.

zoids do not take their posterior sacs with them into the archegonium, but leave them in the mucilage in front of the opening. Sometimes the spermatozoids are so numerous that they crowd between each other and fill up the whole neck-canal with a filamentous mass and form besides a tuft about the opening.

Finally, we will examine the archegonium in section. Make a median section of the prothallium. This may be facilitated by laying several of them together after first carefully removing every adhering grain of sand. The archegonium is, as we see in Fig. 96, *A* and *B*, provided with a ventral part embedded in the prothallium, and a

curved neck-portion. The neck-canal cells, K^7 , and the ventral-canal cells, K^9 , are now distinguishable. So also the ovum, o , with its nucleus. The ventral part of the archegonium is inclosed in a layer of flat cells. In the ripe, opened archegonium, B , at the apex of the ovum, there is often seen a colorless place, the embryo sac, where the spermatozoid is received. Other sections not median will give us a sectional view of the antheridia.

The *Selaginella* are heterosporic *Lycopodiaceæ*. They have two kinds of spores and sporangia, which we will now examine in order to complete our view of the vascular cryptogams. The *Selaginella* are also called ligulates because their leaves are provided at the base with a small tongue. Take *Selaginella Martensii* Sprg., a greenhouse plant. The fertile examples are easily recognizable by the spikes or ears borne on the terminal branches. The vegetative body of the plant is spread out in a plane, and bears four rows of leaves in pairs which obliquely cross. In each pair the upper leaf is small, the lower considerably larger. The two series of upper leaves on the back side press against the stem with their upper side. The two series of under leaves on the ventral side are spread out flat laterally, with their upper side up. The vegetative body of the plant is therefore bilateral and dorsi-ventral, that is, there is but one symmetrical plane in which the plant is laid out in a right and left half, and with a dorsal and ventral surface. The fertile terminal spikes are four-sided and provided with four rows of leaf-flets of like form turned upwards. We study the structure of the spikes in this way. Putting it under the simple and beginning at the bottom we take off one leaf after another with a needle, in the axil of each of which we find an oval, somewhat flattened sporangium. We soon see that many of the sporangia are larger than the

others and are provided with a projecting knob. If we open one of these sporangia with a needle, four large spores which perfectly fill the sporangium, and whose walls are sometimes arched, make their appearance. If we open one of the smaller sporangia we shall find it filled with numerous small spores. The larger are female sporangia, macrosporangia; the large spores, female spores, macrospores. The smaller are male elements and are called microsporangia and microspores. The smaller spores are mostly produced in fours, and have three flat surfaces which come to a point on one side; on the other, or rounded side, the wall is beset with netlike ridges. We meet the same relations in the macrospores, correspondingly larger. The walls of the microspores soon become dark brown while those of the macrospores remain much clearer. If we observe the leaves from which the sporangia have been removed, we shall see the ligula as tongue-shaped membranes close over the place of insertion of the sporangia. A further removal of the leaves from the spike shows us that the macrosporangia are much less numerous than the microsporangia, and are principally confined to its lower part. The ripe sporangium opens transversely with two lips.

Herbarium specimens soaked out may be used for the study of the vegetative cone and the sporangia. Sections of either fresh or soaked material are made beautifully transparent, by the use of potash lye.

NOTES.

- (1) See Pfeffer, *Unters. a. d. bot. Inst. zu Tübingen*, Bd. I, p. 370
- (2) The same work, p. 360.
- (3) *Strasburger, Jahrb. f. wiss. Bot.*, Bd. VIII, p. 405.

LESSON XXVII.

THE REPRODUCTION OF THE GYMNASPERMS.

THE phanerogams are divided into two large groups, those having naked seeds and those having covered seeds, the gymnosperms and the angiosperms. These groups are distinguished by differences in the structure of the flower, and in the processes of fertilization and germ-building, which we will consider first of all in the gymnosperms. We shall make acquaintance first with the structure of the male flower (1) of the fir tree, *Pinus sylvestris*. It ripens the pollen towards the end of May. Alcohol material may, however, be successfully used, but on account of its brittleness it should be soaked out a few days before using in a mixture of like parts of alcohol and glycerine. Material thus prepared makes far better sections than when fresh. We first observe that the male flowers are placed in large numbers on the under parts of contemporaneous or growing shoots. They are arranged in a $\frac{1}{2}$ order, and correspond in position to that of the two-needle branches which adjoin the blossoms in an interrupted series. The blossoms like the leaf bundles occupy the axils of the secondary leaves or scales. Three decussate pairs of scales are found on the style of the male flower. The lower pair is laterally placed in relation to the covering scale and the mother-shoot, a position determined by the existing space relations involved, a position the reverse of that occupied by the first pair of leaves of the vegetative buds of the gymnosperms, almost without exception. Next to the scales of the short style come the stamens, closely compressed and mostly arranged in ten

regular series. The axis of the blossom is elongated spindle shape. A single stamen removed and examined with the simplex appears round, the under side occupied by two pollen sacs longitudinally inserted and meeting along a median line; at the apex runs a short seam which extends upwards. A median, longitudinal section through the blossom shortly before the flowering, Fig. 97, *A*, and



FIG. 97. *Pinus pumilio* agreeing with *Pinus sylvestris*. *D* from *P. sylvestris*. *A*, longitudinal section of a ripe male blossom. $\times 10$. Longitudinal section of a single stamen. $\times 20$. *C*, transection of a stamen. $\times 27$. *D*, a ripe pollen grain. $\times 400$.

treated to potash lye, will show the course of the vascular bundles in the axis of the flower, the single vascular bundles with which each stamen is provided and the insertion of the pollen sac on the stamen.

In less perfect sections thin places will be found where the structure of single stamens may be still better made out, *B*. By making a tangential section of the blossom,

we get a transection of single stamens, *C*, which we will take for more exact study. We see that the two pollen sacs come together in the middle and when ripe are separated by a flat wall of compressed cells which are finally intercalated by one or more layers of flat cells containing starch. The pollen sacs are covered with the epidermis on their free surface, on the inside of which are mostly compressed cells. In the middle of the stamen, in the upper and under part of the wall which separates the two pollen sacs, runs a mesophyll stripe. The upper is the larger and is penetrated by a very delicate vascular bundle. On the lateral edges of the stamens the epidermis projects in the form of minute wings. If they are sufficiently large they contain a little mesophyll. On the under side of the pollen sacs the epidermal cells diminish in size from both sides, and at the point of least development the sac opens. These pollen sacs very closely resemble the sporangia of the *Lycopodiaceæ*. In fact, the recent investigations in comparative biology have led to the conclusion that the pollen sac of the phanerogams and the microsporangia of the cryptogams are homologous forms. If we now examine a pollen grain produced in this sac, in as fresh a condition as possible, we shall see that it has a central body on which are fixed the two lateral sacs, *D*. In the ripe blossom these will appear black being filled with air. Very pretty markings are seen on the surface. The inside of the central pollen grain proper is filled with a finely granular protoplasm and a large nucleus. Shortly before the blossoming, that is just prior to the opening of the pollen sac, the pollen grain is divided by a wall, shaped like an hour-glass, which forms a lens-shaped cell on the posterior portion; that is, on the side turned away from the point where the wings are inserted. This cell is best seen when the grain lies on its side as in our figure. A cell quite like this is differentiated in the mi-

crosspore of the heterosporic *Polypodiaceæ* before the beginning of the process of development which leads to the formation of the sexual product. There it is considered a vegetative cell and may be so designated here. The wings of the pollen grain are produced late as the developmental history teaches, by the elevation of the cuticle between which and the inner thickening layer of the wall a watery fluid collects.

We will next take the male flower of *Taxus baccata*. It opens in March, but by means of alcohol material one may examine it at any time. The male flower is found in the axils of the leaves of last year's branches. It begins with some decussate pairs of scales which pass over to the $\frac{2}{2}$ arrangement. The scales grow constantly larger and soon fall into a quite indefinite arrangement on the elongated axis of the shield-shaped stamen. The whole bloom as seen with a magnifying glass resembles not a little the fertile sporangia-bearing leaves of the Equisetum. By removing a stamen and examining with the simplex, we shall find that beneath the shield are inserted from five to seven pollen sacs. These have their base affixed to the under side of the shield, and their inner side to the style. Laterally, they are mainly free next each other, and wholly so outwardly and at their apex. Make a median and also a tangential longitudinal section; the former will show us the stamen and pollen sac in longitudinal section and the latter in transection. The pollen sac widens outward, the section showing a wedge-shaped form. Both sections show that the walls of the ripe pollen sac are reduced to the epidermis and a layer of compressed cells. The walls of the epidermal cells are provided with thickened ledges, and when the pollen sac is separated from the style the epidermal cells show a considerable reduction in size. By removing a pollen sac wall from the stamen, with a needle, we shall see that the thickened

ledges on the inner and side walls of the epidermal cells are U-shaped. This thickening occurs also on the epidermal cells of the outer surface of the shield. The pollen sac is opened by the walls parting from the style and stretching. The pollen grains are ellipsoidal in form and beset with small knobs. Shortly before flowering, the end of the grain is differentiated into a small cell. In alcohol material the contents of the pollen grains are shrunken and unserviceable for the investigation.

The pollen grains of *Taxus* are not provided with the bladderly inflations of the walls observed in the *Pinus*, nor do they occur in all the *Abietinæ*; but, on the contrary, they recur again immediately below the *Taxus* in the *Podocarpus*. In many genera, more than one vegetative cell will be differentiated from the contents of the pollen grain whereby projecting cell bodies will be produced within the grain. Among the *Abietinæ* only the genus *Pinus* has simple vegetative cells.

The female flowers of the *Taxus baccata* (2) are found on other individuals, since the plant is diœcious, and like the male flowers in the axils of the leaves of last year's branches, Fig. 98, *A*. It blossoms, as we already know, in March, but alcohol preserves the blossoms very well, and such specimens serve the purpose of our investigation perfectly and are easily managed if they are permitted to lie in glycerine and alcohol for at least twenty-four hours. The blossoms apparently terminate a small shoot, but are in reality not terminal. Not seldom we find two flowers on the same shoot, Fig. 98, at *. In rare cases one meets with a malformation in which a growing foliage shoot springs out of the side of the blossom, Fig. 98, *B*. With a magnifying glass we shall perceive that the floral shoot begins with a lateral pair of scales upon which follow in a spiral order other scales which gradually increase in size. The blossom itself is inclosed in three decussate pair of

scales from which only its protruding apex is seen. This apex shows a punctiform opening, the micropyle. We must carefully arrange the shoot in order to get a median longitudinal section. The section should be made through the middle of the pair of scales which stands next but one beneath the blossom. We should select for our ex-



FIG. 98. *Taxus baccata*. *A*, typical form of a branch with female flowers at the period of fertilization; at * are two ovules on the same primary shoot, natural size. *B*, a leaf with a floral bud in its axil, the primary shoot being turned aside. $\times 2$. *C*, a longitudinal section through the common middle of a primary and secondary shoot; *v*, vegetative cone of the primary shoot; *a*, beginning of an axillus; *e*, beginning of an embryo-sac; *n*, nucellus; *i*, integument; *m*, micropyle. $\times 18$.

amination a blossom towards the end of April, somewhat old and already pollinized, as it will be easier to cut and will in many respects be more instructive. If the section is made in the direction indicated, the image will be like that represented in Fig. 98, *C*. The blossom appears

not to be terminal on the primary shoot, this having terminated its development by the formation of a minute, secondary shoot in the axil of its uppermost scale, which shoot ends in the flower after it has produced three decussate pairs of scales. Laterally, from the insertion of the secondary shoot, is seen the vegetative cone, *v*, of the primary shoot, pressed to one side. Here and there, the next scale but one to the last on the primary shoot forms a secondary shoot, which terminates with a blossom; and, in rare cases, as we have seen, the primary shoot forms foliage leaves, Fig. 98, *B*. The pairs of scales which precede the blossom are to be looked upon as its foliage envelope, the blossom itself being reduced to an ovule. We see one of them in the form which terminates the secondary shoot. We distinguish in a longitudinal section the following parts: the envelope, *i*, with a small opening at the top of the micropyle, *m*, and within this the so-called bud-nucleus, the nucellus *n*, in the bottom of which under favorable conditions, by treatment with potash, we may recognize a large cell, the beginning of the embryo sac, *e* (3). As the pollen sac corresponds to a microsporangium, so the ovule corresponds to a macrosporangium, the pollen grain to a microspore and the embryo sac to a macrospore.

Biological investigations (4) have discovered that there are important resemblances in the beginnings of these forms; still at the same time showing that a progressive reduction befalls that which in the phanerogams lead to the first beginnings of the macrospore. On the other hand, there are no grounds for comparing the integument with the indusium of the vascular cryptogams. The integument is a new formation appearing on the macrosporangium of the phanerogams. On the style of the ovule of *Taxus* is seen a small tissue-mound, *a*, which remains stationary

for a long time, till into June, but afterwards begins to grow and in the fall forms the bright red arillus which covers the ripened seed. On the already pollinated bloom which we have taken for our investigation, we may find, lying at the apex of the nucellus, a pollen grain, which has driven a short tube into the tissue of the apex.

It is the large cell of the pollen grain which is grown out into this tube, while the small vegetative cell is shrunken up. The inner covering of the pollen grain, the intine, forms the pollen tube, while the outer integument, the warty covering of the ripe pollen, the exine, will be stripped off. The pollen grain lies here on the papillose surface of the apex, while in other species of *Taxus* and its near relations, the apex is hollowed out (5) to receive the pollen grain, forming the so-called pollen cavity. If we would learn about the contrivance by which the pollen grain is brought into the ovule, we must make our observation in nature, during the flowering-time of the plant (6). If we examine a female plant at the time the pollen is ripe and being discharged from the pollen sac, we shall see that each of these blooms secretes a little drop of fluid from its micropyle. The pollen grain, borne by the wind, falls into this drop of fluid and is sucked in with it.

The fir, *Pinus sylvestris*, will give us another and an extreme example of the structure of the pistillate blossom in the conifers. It being monœcious, both forms of flowers are found on the same plant. The ovule does not, as in the *Taxus*, stand alone, but a cone is produced in which numerous buds are united together, inserted on scale-like processes. The small cones occupy the tip of the present year's shoots, singly or in clusters. They stand in the axils of the bracts, like the lateral, two-nedded branches inserted below; but their position above, on

the shoot, corresponds to that of the branch forming long shoots. The small cones are for the most part capable of fertilization by the end of May and are noticeable in their relatively smaller size by their brown-red color. They are borne upright upon a stem, the stem being covered with brown scales. Use alcohol material which has been treated with glycerine for the investigation. Cut away the separate parts from the axis of the cone with a scalpel and examine them under the simplex separating them out with a needle for the purpose.

It will be seen that standing in the axils of the delicate, reversed-oval, enveloping scales with a fringed edge, Fig. 99, *b*, are similarly shaped, more thickened, smooth-edged scales, *f*, provided on the inner surface with a median projecting carina, *c*. The latter are the seminiferous scales. At the right and left of these scales, at the bottom, is inserted an ovule with the micropyle of each directed downward and outward. The edge of the integu-

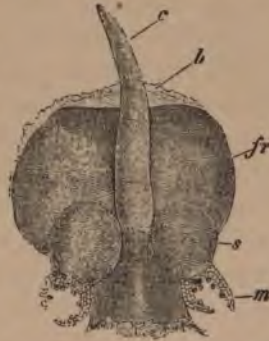


FIG. 99. *Pinus sylvestris*. Seminiferous scale *fr*, with the two ovules *s* and the keel *c*; behind is the covering scale, *b*. On the ovule the integument is grown out into two processes, *m*. $\times 7$.

ment of the micropyle is elongated into two right and left flaps, *m*. Bracts and seminiferous scales grow together at the base and so remain attached to each other when separated from the axis of the cone. The cones of *Abietinae* and other conifers will be considered as single flowers or as a mere receptacle for flowers according to the significance which one attaches to the seminiferous scales. They must be considered either as flattened metamorphosed axillary shoots partly grown to a bract, or as a placental growth of a carpel which has heretofore been known as an

enveloping scale. In the first case, we treat each as a shoot in the axil of the bract, bearing two ovules; in the other case, we consider it as a placenta bearing two ovules, placed on the upper side of its carpel. In the first instance, the inflorescence would consist of a cone composed of many fertile, axillary shoots, and in the other the cone would be a single bloom formed of numerous carpels.

The remarkable structure of the seminiferous scale is explained in reference to the act of fertilization and so can be followed out only in fresh material at the time of pollination (7). As soon as the production of pollen begins in the male blossom, one will notice an elongation of the axis of the little cones, by which the seminiferous scales and the bracts which belong to them are separated a little. The pollen may now fall upon the erect seminiferous scale, slide down, and, guided by the carina, come at last between the two processes of the integument. These subsequently roll up and conduct the pollen grains into the micropyle and to the embryo sac. After being fully pollinized the growing seminiferous scales soon glue their edges together with resin. Neither the bracts, nor the carina develop farther, the latter having no further function. The red color of the cone passes into brown and finally to green, the cone gradually taking a hanging position.

We shall next examine another variation in the development of the pollinized ovule of the conifers (8). We have already learned that the time of pollination for the embryo sac of *Taxus* is in the first beginning of it. From this followed a further development of the ovule so that a considerable length of time elapsed between the pollination and the fructification of the ovule. In *Taxus* the fructification takes place in the middle of June of the same year; in the fir, not till the next year, thirteen

months after the pollination. In the pine, the two acts are separated by but about six weeks. We shall use the fir for our investigation. It would lead us too far to follow step by step the development of the embryo sac, the beginning of the prothallium tissue, the endosperm, and the reproductive organs in the same, the increase in size and consequent differentiation of the whole ovule. But we will take it at the point when the ovum is fully developed and ripe for fertilization. This condition is reached by the common or red-fir, *Picea vulgaris*, about the middle of June the pollination following in the course of a few days. Alcohol material will be found better than fresh since the ovum will be fixed. It is better to put the separate scales rather than the whole cone in the alcohol; and the material should, as heretofore recommended, be previously treated to a mixture of alcohol and glycerine, equal parts, for at least four and twenty hours before making the sections.

We should first take a general view of the whole scale. It is an inverted oval, on the inner surface of which the beginnings of the two seeds are seen; also the outline of the wings, which afterwards as thin lamella of tissue will be separated from the inner surface of the seminiferous scale. Beneath, on the outer surface of this scale, the bract is still to be found, now, however, quite small. We may easily remove the ovule uninjured from the seminiferous scale with the needle in order to make a section of it. Make the longitudinal section between the thumb and finger. The hardened under portion of the ovule will not so readily lend itself to section-making. So cut away the lower half with the shears and make the section through the soft upper part containing the nucellus and the embryo sac. Staining media are to be used with great caution since they stain the whole protoplasm of the ovum and

easily render it untransparent. First use a low power and as we are looking upon a median section cut at right angles with the base of the ovule or surface of attachment, we shall see the various elements as represented in Fig. 100. The integument, *i*, forms the outer envelope of the ovule

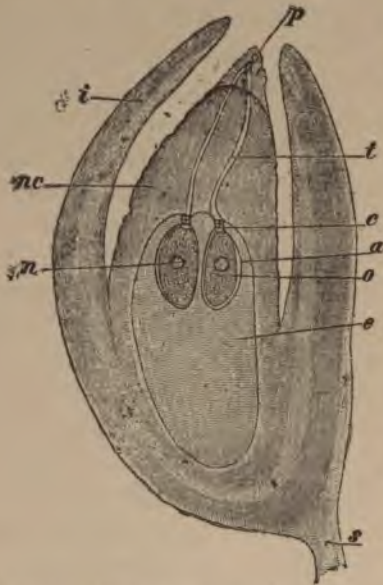


FIG. 100. Median longitudinal section of the fertilized ovule of *Picea vulgaris* Lk. *e*, embryo sac filled with endosperm; *a*, ventral part of archegonium; *c*, neck part; *n*, nucleus of ovum; *nc*, nucellus or kernel of the bud; *p*, pollen grain on and in the bud-nipple; *t*, pollen tube which penetrates the nucellus; *i*, integument; *s*, seed wings. $\times 9$.

and is separated from the nucellus about half way up. The nucellus bears pollen grains, *p*, on its apex, which lie partly within and partly without the tissue. The tubes, *t*, from these pollen grains will eventually penetrate the upper part of the nucellus in order to reach the embryo sac, *e*. The latter is elliptical in outline and filled with endosperm, or more correctly prothallium tissue. The ventral part of the archegonia, *a* (corpuscula) may be easily recognized but not so easily the neck part, *c*. Within each archegonium is an ovum, *o*, distinguishable in alcohol material by its yellow-brown color, in the middle of which is a nucleus, *n*. Finally, the attachment of the seed wings, *s*.

If we make a section through a fresh specimen we shall

and is separated from the nucellus about half way up. The nucellus bears pollen grains, *p*, on its apex, which lie partly within and partly without the tissue. The tubes, *t*, from these pollen grains will eventually penetrate the upper part of the nucellus in order to reach the embryo sac, *e*. The latter is elliptical in outline and filled with endosperm, or more correctly prothallium tissue. The ventral part of the archegonia, *a* (corpuscula) may be easily recognized but not so easily the neck part, *c*. Within each archegonium is an ovum, *o*, distinguishable in alcohol material by its yellow-brown color, in the middle of which is a nucleus, *n*. Finally, the attachment of the seed wings, *s*.

find the same relations again, only that the contents of the archegonium will often be discharged. If the section touches an archegonium without opening it, the ovum will appear as a yellowish, foamy, protoplasmic mass in which the nucleus is scarcely distinguishable or at best has the appearance of a large central vacuole. The ovum soon begins to suffer from the effects of the surrounding water. If it is desirable to preserve the section for a considerable time it is recommended to use diluted white of an egg for an examining fluid, and to make this still more durable a little camphor may be added to it (9). In such preparations, the neck part of the archegonium is not difficult to see. It consists of from two to four stories of cells. Under the neck part is a small cell which corresponds to the ventral canal cell of the vascular cryptogam, the ovum parting to form it shortly before ripening. The ventral part of the archegonium is surrounded by a layer of flat cells rich in contents, like the covering which we saw about the ventral part of the fern. In order to know the number and position of the archegonia we must make a series of transections through the upper part of the ovule. By this means we shall see that from three to five archegonia are arranged in a circle at the apex of the embryo sac. Sections which touch the apex of the embryo sac present us with an apical view of the neck part of the archegonia which is a rosette of from six to eight cells. If our material has been collected at the period of fertilization we shall eventually find pollen tubes which have penetrated to the ovum, and in the under part of single ovules we shall find a four-celled rosette which may be traced out into prothallium tissue in four uninterrupted tubes. The four terminal cells of these tubes produce the germ. The seeds ripen in October. It separates then easily from the seminiferous scale. The wings continue

on the inside of the seed between them and the seminiferous scale, the seed falling off later from the wings, leaving a corresponding cavity in the same. Sections in both directions will show that the cells of the seminiferous scales are so thickened as nearly to obliterate their cell cavity. A part of the prothallium tissue is filled with reserve material which has been preserved as seed albumen or endosperm in the seeds. It forms a sac inclosing the germ. This sac is open on its micropyle end and here the root end of the seed joins the remainder of the sup-
 planted nucellus. The germ appears like a cylinder which grows thicker toward the end of the cotyledon, and in consequence of being filled with albumen is white and untransparent like the cotyledons. Make a longitudinal section between the fingers and examine it in carbolic acid diluted with alcohol. This makes the image very clear, far more so than potash lye or even chloral hydrate itself, so that each row of cells may be easily followed. We see Fig. 101, *c*, that the cotyledons do not reach quite a third of the length of the germ and between them at the base is the vegetative cone of the stem. The little stem or cauliculus which will be designated the hypocotyledon or hypocotyledonous link, *h*, is a posterior continuation without distinct limits of the rootlet (radicle). This is mainly discernible only by means of a vegetative cone which shows itself distinctly within the body of the germ as the apex of the plerome, of the root *pl*, while the cell rows of the rind of the hypocotyledon continue directly into the parabolic layers of the root-cap,

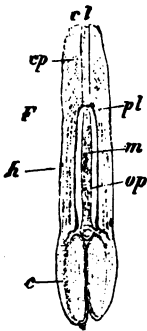


FIG. 101. *Picea vul-
 garis*. Longitudinal
 section of the ripe
 germ; *c*, cotyledon;
h, hypocotyledonous
 member; *pl*, apex of
 plerome; *cp*, root
 cap; *cl*, middle col-
 umn of the same; *m*,
 pith; *op*, procambium
 ring in the hypocoty-
 ledonous member. \times
 10.

the vegetative cone of the stem. The little stem or cauliculus which will be designated the hypocotyledon or hypocotyledonous link, *h*, is a posterior continuation without distinct limits of the rootlet (radicle). This is mainly discernible only by means of a vegetative cone which shows itself distinctly within the body of the germ as the apex of the plerome, of the root *pl*, while the cell rows of the rind of the hypocotyledon continue directly into the parabolic layers of the root-cap,

cp, a process which we find repeated in all roots of the gymnosperms wherever we can see the cell rows of the rind of the root-body pass over directly into the cell layers of the root-cap. See *Thuia*, p. 177. The root-cap is penetrated in its long axis by a pith-like column, *cl*, of cells arranged in straight rows. In the hypocotyledon, the tissue of the pith, *m*, already begins to show itself, and about this the elongated cells of the procambium ring, *op*, in which the vascular bundles appear. These cells may be plainly seen in a median section of the cotyledon. See the figure. Thus we see that the essential parts of the future plant already appear in the embryo.

NOTES.

- (1) See Strasburger, Coniferen u. Gnetaceen, p. 120; Eichler, Blüthendiagramme, Bd. I, p. 58; Goebel, Grundzüge, p. 363.
- (2) Strasburger, Coniferen u. Gnetaceen, p. 2.
- (3) Strasburger, Angiosp. und Gymnosperm, p. 109.
- (4) Strasburger, same work, p. 109; Goebel, Bot. Ztg., 1881, sp. 681.
- (5) Strasburger, Jenaische Zeitschr. f. Naturw., Bd. VI, p. 251; Conif., u. Gnet., p. 250.
- (6) Same work, pp. 250 and 265.
- (7) Strasburger, last work, and Vol. quoted, p. 251; Conif. u. Gnet. p. 267.
- (8) See Strasburger, Befr. b. d. Conif.; Conif. u. Gnetaceen, p. 274; Befr. u. Zellth. a. v. O. Angiosp. u. Gymnosp., p. 140; Goroschankin, Ueber die Corpuscula u. d. Befr. bei d. Gymnosp. russisch., 1880.
- (9) Strasburger, Befr. b. d. Conif., p. 8.

LESSON XXVIII.

THE ANDRŒCEUM IN THE ANGIOSPERMS.

THE collective male organs of an angiosperm blossom form the andrœceum. The pollen vessel or pollen leaf (stamen) (1) consists of a filamentous support, the filament, and the anther. The latter is formed of two parts lying side by side lengthwise and separated by the upper part of the filament, the so-called connective tissue. This tissue it is best to reckon as a part of the anther. Two pollen sacs are commonly embedded in the tissue of each half of the anther. Each of these sacs or compartments correspond to a microsporangium. For a study of the stamen we will take in the first case a large flowered lily, as for example, the *Hemerocallis fulva* cultivated everywhere in gardens. The yellow filament is very long and becomes slenderer above and is very sharply pointed at the place of insertion in the anther. The anther is brown and movable on the filament. The connective tissue may be traced as a narrow stripe on the outer surface of the anther between the two lobes. The ripe pollen may be examined on the slide. It has the form of a coffee bean. It is yellow and its surface is covered with reticulated ledges. If a little water be introduced from the side of the cover-glass, the crease or fold in the pollen grain will soon disappear and the grain on that side swell out till it takes the form of an ellipsoid flattened a little on one side. The membrane of the before-infolded part is relatively of considerable thickness, colorless, without markings and is very sharply defined against the brown-

ish-marked membranous part. By careful focussing on a favorably placed pollen grain we learn that it is enclosed in a simple membrane, that the colorless part thins out at the edges and passes directly into the colored part. Between the grains in the preparation and also adhering to their surfaces are orange-red oil drops which in their dry state give them their yellow color. The contents of the pollen grain are gray and finely granular. After a short time, during which the grain gradually enlarges, it bursts and empties its contents vermiformly in the surrounding water. If a solution of sugar of the proper concentration be used the pollen grains will round out and not burst and may be examined unhindered. Treating with concentrated sulphuric acid, the smooth, colorless portion of the wall slowly dissolves, but the striated colored portion resists the action of the acid; it is cutinized. The cutinized membrane of pollen grains which have an infolded part protects the whole grain when the anther is open. As may be seen in the dry grain the edges of the cutinized portion of the membrane touch each other along the whole length of the fold so that the uncutinized part lies wholly buried under it in the fold. It comes to view only when the pollen grain is placed on the stigma, begins to swell and puts out the pollen tube. But in the pollen of this plant an exine and intine or special outer and inner part is not to be distinguished, since the wall is nowhere double. Its cutinized part has the function of exine, and the uncutinized of intine in other pollen grains. By the action of sulphuric acid the structure of the cutinized membrane is very distinctly seen. By strong magnification and looking at it from above, we see a wandering network with delicate wavy walls. In some of the meshes lies a blue irregularly shaped body which is the oil drop, before yellow but now colored blue by the acid. The cu-

tinized membrane itself is colored yellow. By a median focus it is easy to see a compound inner wall-layer on which rest the outer projecting ledges. The ledges themselves are swollen on their outer edges so that in optical transection they appear club-shaped. In a superficial view we find that the ground surface is covered with fine points the optical transection of which demonstrates them to be in reality small knobs resting on the inner wall-layer.

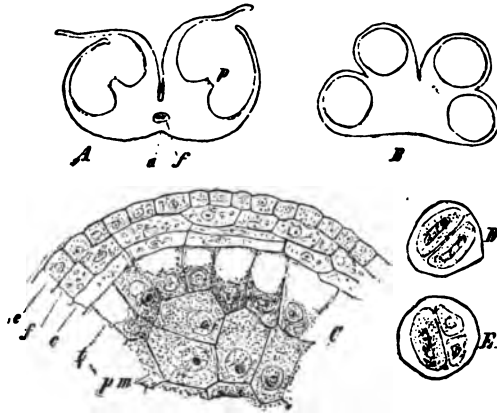


FIG. 102. *Hemerocallis fulva*. A, transection of an almost ripe anther, the cutting having opened the pollen chambers; *p*, the division walls between the compartments; *f*, vascular bundle in the connective; *a*, furrow in the connective. $\times 14$. B, cross-section of a young anther. $\times 28$. C, part of the last preceding section, the tissue over a chamber; *e*, epidermis; *f*, cells which afterwards form the fibrous layer; *c*, a cell layer which will disappear; *t*, tapestry layer which afterwards dissolves; *pm*, pollen mother-cells. $\times 240$. D and E, pollen mother-cells which have undergone self-division. $\times 240$.

After being subject to the action of the acid for some hours the pieces of the membrane take on a reddish-brown color while the contents of the pollen grain are at the same time tinged a rose-red, a behavior which protoplasm often shows towards sulphuric acid (2). In 25% chromic acid the uncutinized membrane and the contents of the grain are rapidly dissolved, the cutinized part resisting longest,

the network of ledges alone remaining and these finally disappearing.

We will now make a transection of the anther which we can do best by taking a two-thirds grown flower-bud and cutting through the whole of it. With the needle remove the sections of the perigoneal leaf from the preparation. Notwithstanding we have taken so young a flower, we shall find all the compartments of the anther open. They open very easily and the pressure of the knife in cutting the section has done it. Figure 102, *A*, represents the section. The walls of the two pollen chambers separate themselves from the division wall at *p*. They unfold their bent forms. The two halves of the anther are joined together by a slender connective containing a vascular bundle, *f*. Examined with a high magnification, we find a flat-celled epidermis on the outside, filled with violet cell-sap. These epidermis cells are bowed outward. On the edges of the chamber walls the height of these cells rapidly diminishes, and at this point the middle division wall ruptures. Stomata are distributed over the whole surface of the anthers, a small breathing cavity lying under each. Next succeeding to the epidermis on the walls of the compartments is a single layer of relatively high cells provided with ring-shaped thickenings, the so-called fibrous layer. The rings of these cells are placed perpendicular to the surface, sometimes pass into spirals and anastomose in many ways with each other. Towards the back of the anther the walls of the compartments become gradually thicker, the layer of fibrous cells being doubled. The rest of the body of the anther is also built of these fibrous cells. Only those cells which surround the vascular bundle in the connective and those which form the division walls between the chambers of the anther, *p*, are without thickened ledges.

We must take a two-thirds grown flower-bud in order to get a good superficial section of the anther. This will show us that the epidermal cells are elongated longitudinally over the compartments while those of the fibrous layer are extended perpendicular to the surface. But on the back side of the anther the fibrous cells are more nearly isodiametric. Over the pollen chambers the thickened ledges of the fibrous cells are much weaker on the outside of the walls, often scarcely discernible, while the lamellæ of the thickened ledges on the inside next the cell space are drawn closer together than those of the outside by drying. This causes the walls of the compartments to spring back when they are ruptured. Frequently, in the angiosperms, as in *Taxus*, the thickenings of the outer walls of the fibrous cells in the walls of the compartments cease altogether, so that the ledges become U-shaped or basket-shaped, opening outward. It is clear that such an arrangement facilitates the rolling back of the tissue forming the compartment walls. In order to understand the relation of the filament to the anther, we must make a median longitudinal section of the upper part of the stamen between the two halves of the anther. We see that the filament is very much attenuated at the point where it is inserted in the anther. The cells which surround the vascular bundle may be traced from the filament into the connective. They are not fibrous cells. In order to get a section of the anther with its chambers closed, Fig. 102, *B*, we must take a sufficiently immature flower-bud for our section.

If now we make a transection of a floral bud only 6 or 7 mm. high we shall find in the walls of the chambers besides the epidermis, Fig. 102, *C*, *e*, two or three layers of flat cells, *f*, *c*, and one of radially elongated cells, *t*. The latter inclose the whole chamber, the interior of which is filled with polygonal pollen mother-cells, *pm*.

By making a section of a bud 1 cm. long, we shall find the pollen mother-cells already isolated and in the act of self-division. They are recognizable by their thick, white, strongly refractive walls, their contents being already divided into from two to four cells which lie in one plane, Fig. 102, *D*, or in two planes at right angles, Fig. 102, *E*. These become subsequently the pollen grains, produced, like the spores, by fourfold division within their mother-cell. The walls of the anther are lined with so-called "tapestry cells", *t*, which are filled with yellow-brown contents, and arise from the innermost of the layers lining the chamber. In the next older flower-bud the walls of the pollen mother-cell are dissolved, the young pollen grains lie free, the tapestry cells have for the most part given up their independent existence, their contents being pressed in between the young pollen grains. The layer of flat cells, *f*, lying immediately beneath the epidermis is much developed and forms the fibrous layer, while the next inner layer has become compressed and disorganized. An older bud will show that the still unused tapestry cells, especially in the periphery of the chamber, have an intense yellow-brown color, and oily sparkling appearance, and thus form the oily substance which surrounds and adheres to the pollen-grains.

All species of lilies behave like the *Hemerocallis*, but the differentiation of the anther comes later. The pollen mother-cells begin to divide in *Lilium candidum*, *L. croceum* and other species, only when the flower bud is 2 cm. high. In transections of fresh buds the large tapestry cells will be very noticeable with their yellow-brown contents. The hypodermal cells as well as all others which are afterwards to be provided with ledge-like thickenings are filled with starch grains.

Funkia ovata is a good plant for study, and behaves like *Hemerocallis* and *Lilium*; so also *Agapanthus umbellatus*.

Tulipa and *Hyacinthus orientalis* are likewise good objects. In *Tulipa* the filament is so much attenuated under the anther that the latter will rotate. In the hyacinth the anther almost sits on the perigone.

Tradescantia Virginica is less easy to make sections of, but we will examine it in reference to the pollen grains. A transection of a bud about two-thirds grown shows us the two halves of the anther separated by a rather thick connective. The walls of the compartments are already reduced to two layers of cells, and the thickened ledges are already developed on the inner one. The pollen grains are embedded in a yellow-brown substance

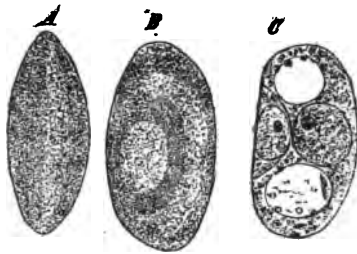


FIG. 103. *Tradescantia virginica*. A, pollen grain dry; B, in water; C, young pollen grain in water showing the vegetative cell. $\times 540$.

with whose origin from the tapestry cells we are already acquainted. The division wall between the two chambers is so fully developed and is so thick that there is scarcely any depression observable between them. At the point of insertion of the chamber wall upon

the division wall the fibrous layer suddenly ceases, and at this point the separation takes place later on. An examination of the surface of the chamber wall shows in this case a longitudinal diminution of the epidermal cells and a transverse lessening of the fibrous layer with a most complete failure of the thickened ledges on the outside walls of the cells.

If we examine with the magnifier the stamen of a bud just ready to blossom out, we shall see the beautiful yellow anther on the violet colored filament beset with violet hairs. The dry pollen grains are now folded on one side, Fig. 103, A. In water, the fold is smoothed out

and the grain becomes ellipsoidal in shape, the previously folded side being more convex than the other. The membrane is striated with meandering lines. The folded side shows this structure also and is distinguished only by its brighter color and its somewhat weaker cutinization. In the finely granular contents are to be distinguished two clear, homogeneous spots, *B*. They are the two nuclei, of which one is vermiform and the other ellipsoidal. The rest of the contents of the pollen grain is pretty uniformly fine-grained. The pollen grain very soon begins to flatten, whereby the nuclei together with the rest of the contents are much compressed. Both nuclei may be seen very beautifully if the pollen grain be crushed in a drop of acetate of methyl green, or acetate of iodine green. The vermiform nucleus is deeply stained and much elongated, after its exit from the pollen grain. If the pollen grain be put in the staining fluid without being crushed, the nuclei will be seen in their natural position, the vermiform one somewhat more deeply stained than the other. The rest of the grain will remain uncolored. If the pollen grain be crushed in a drop of water to which has been added a solution of potassium iodide of iodine, numerous small blue starch granules will be seen among the yellow-brown contents. (3)

If we now return to the young flower-bud and take one about 6 mm. long and crush the anther in water, we shall find that part of the grains have but one nucleus and others two, lying close together as in Fig. 103, *C*. The two nuclei are separated by a wall which incloses one of them, together with a little protoplasm. This flat cell which is almost circular in form always lies on the flat side of the pollen grain where the fold in the membrane is afterwards found. In a somewhat older bud this cell is found to be separated from the wall of the pollen grain and lies free in the contents of the grain.

The pollen grains have here become longer and correspondingly slender and pointed at the ends. With the exception of the two ends they are filled with this nuclei. (4). In nearly ripe pollen grains the definite demarcations of the nuclei disappear and they lie free in the grain more or less elongated in a vermiform shape.

In comparison with the gymnosperms, to which they lie very near, we should hold the small cells to be vegetative; but really it is the generative cells with their highly stainable nucleus which are concerned in the fertilization. The difference in the staining quality of vegetative and generative nuclei is far more striking than in *Tradescantia*. We may make the above described examinations, in the case of the younger pollen grains in pure water, but in the older stages we must use methyl green or iodine green with acetic acid. The species of *Lucojum* act quite like the *Tradescantia*.

If one opens a bud of *Œnothera biennis* which is about ready to blossom, he will find that the anthers are already open and the pollen escaped. Afterwards viscous fibres are seen between the anthers. Putting one of these on a slide, it appears under the microscope an extremely delicate thread partly stretched out straight and in part wavy. The pollen grains when dry are untransparent but their triangular form is apparent. In water and with a higher power we see that they are flattened, equilateral triangular bodies with warty projecting corners. At the base of each of these warts is to be seen a ring-shaped thickening of the pollen membrane. The contents of the ripe pollen grain are finely granular, and the two nuclei are seen with great difficulty. The pollen membrane is stained a red-brown with sulphuric acid. The acid causes a very thin yellow layer to be lifted up upon the body of the grain, forming folds from an inner, thicker, red-brown layer. Both membranes are united in the walls of the warts. From the lateral walls

of the warts fine teeth project toward the inside so that these walls appear to be porous. The apex of the wart is dissolved by the acid. The fine filaments which connect the pollen grains are insoluble in water, alcohol, potash lye and sulphuric acid. In 25% chromic acid, the pollen membrane dissolves, the strongly cutinized parts rather before the others, the latter being the caps upon the projecting warts, which remain colorless and swollen. These finally dissolve and even the viscid filaments between the pollen grains cannot withstand chromic acid. A pollen grain taken from the stigma of an old flower will show the pollen tubes already grown out, commonly from only one wart, but if from another also, then only just outside the latter. The membrane of the pollen tube passes into the lateral walls of the wart. An intine layer as distinguished from the outer membrane does not occur (5). Instead of *Oenothera* one may use *Epilobium* or *Fuchsia*.

We will now examine some peculiarly formed pollen grains. Those of the *Malvaceæ* are of extraordinarily large size. The pollen of *Althæa rosea* in water are globular, untransparent and beset with colorless spines. They become beautifully transparent in carbolic acid, and in chloralhydrate, less so in oil of cloves, still less in lemon oil. The best preparation is with carbolic acid and so we will use that. A superficial view shows us that the colorless pollen membrane is beset with large pointed spines at nearly uniform intervals. Between those are others; short, blunt and of varying thickness. Regularly distributed circular openings appear in the membrane. The surface of the membrane is finely dotted. The contents of the grain are uniformly finely granular, and the nucleus is made out with much difficulty. An optical transection of the grain shows us clearly the form of the

large and small spines and the canals which perforate the membrane. An extraordinarily delicate but really existing intine may be traced only as the outline of the contents. It is papillately arched a little in the canals of the exine. In concentrated sulphuric acid the exine soon stains a red-brown and shows its structure then very distinctly.

Most of the pollen grains of the *Malvaceæ* act like those of *Althæa*. In *Malva crispa*, a frequently cultivated species, the pollen grains are like those of *Althæa*, except that the spines are all alike. Between the spines are distributed the openings in the membrane, the rest of which appears finely dotted.

The large pollen grains of the *Cucurbita* species have long received special distinction on account of the cover which closes the opening in the exine. In water, yellow oil drops exude from the surface of the exine, the grain soon becomes empty of its contents and the structure of the membrane may then be distinctly seen. The exine is beset at regular intervals with large, and between these with many small spines. The openings are round. The cover is lifted up on one side or all around, by the papillately arched intine. It has the structure of the adjoining exine and bears one or more spines. Very good preparations are got by using lemon oil, less serviceable ones in oil of cloves, but those in chloral hydrate and those in carbolic acid are preferable. In each case that medium most suitable for clarifying it is to be sought for. By optical transection in lemon oil or chloral hydrate preparations we are able to demonstrate the position of the cover within the exine, and find it widened inward somewhat towards the base. Under the cover the swellings of the intine may be seen. The oil drops on the exine are col-

ored blue with sulphuric acid. The exine becomes gradually brown. The cover is pushed off by the swelling contents. In 25% chromic acid the whole pollen membrane is soon dissolved, but the intine withstands it longest and is, at the moment where the exine disappears, clearly discernible as a greatly swollen homogeneous membrane. The pollen grain has previously become empty, which facilitates the examination of the intine. In sulphuric acid, on the contrary, the intine is immediately dissolved, while the exine remains and the exuding contents of the grain gradually assume a rosy tint as in other cases.

Of compound pollen grains, which occur both in mono- and dicotyledons, we will look at those of *Calluna vulgaris* first. The grains are united into fours and mostly tetrahedrally grouped. The pollen membrane shows but small protuberances and mostly but three openings to each grain. The species of *Erica*, *Azalia* and *Rhododendron* are essentially the same as those of *Calluna*. In the *Acacia* species, especially in *Mimosa* (6), the pollen grains form groups of four, eight, twelve, and sixteen, or even more.

In a sugar solution of from 3 to 30% which contains 1.5% gelatin, most pollen grains will put out three tubes, in which the streaming of the protoplasm may be beautifully seen. The formation of pollen tubes takes place rapidly and surely in a 5% solution of sugar with 1.5% gelatin with pollen grains from the *Peonia*, *Staphylea* and also when they are taken from a freshly opened flower of *Tradescantia*. The most favorable objects are furnished by the species of *Lathyrus* in 15% sugar solution with 1.5% gelatin. The solution must be freshly prepared and the experiment is best made in a hanging drop in a moist chamber. See page 230.

NOTES.

(1) For stamens and pollen, see v. Mohl, Ueber den Bau und die Formen der Pollenkörner, 1834; Fritsche, Ueber den Pollen, Mém. de sav. étrang, 1836; Naegeli, Zur Entwicklungsg. d. Poll. bei den Phan., 1842; Schacht, Jahrb. f. wiss. Bot. Bd. II, p. 109; Warming in Hanstein's bot. Abh. Bd. II, Heft II; Strasburger, Befr. u. Zellth., p. 15 und Bau der Zellhäute, p. 86; Elfving, Jen. Zeitschr. f. Naturw. Bd. XIII, p. 1; Goebel, Grundz. d. Syst., etc., p. 398; Luerssen, Grundz. d. Bot., III Auf., p. 359; Med. Pharm. Bot., Bd. II, p. 198; Prantl, Lehrb. d. Bot. IV Aufl., p. 192. In the above quoted works is the rest of the literature.

(2) Sachs, bot. Ztg., 1862, p. 242.

(3) Warming in Hanstein's Bot. Abh. Bd. II, Heft II; Goebel, Grundzüge, p. 409.

(4) See also Elfving, Janaische Zeitschr. f. Naturwiss. Bd. XIII, p. 12.

(5) Strasburger, Bau d. Zellh. p. 95. There also the history of its development.

(6) Rosanoff, Jahrb. f. wiss. Bot. Bd. IV, p. 441; Engler, the same periodical Bd. X, p. 277. There also the literature.

LESSON XXIX.

THE GYNECEUM OF THE ANGIOSPERMS.

WE will now take a general survey of the structure of the ovary (1) using the *Delphinium ajacis* or garden larkspur for our purpose. Take an old flower from which the petals and stamens may be easily removed, and notice the three pistils which remain standing in the middle. We shall see first the green swollen part, the ovary, the slender rose-colored portion into which the ovary narrows itself, the style. This finally ends in the stigma which is not in this case especially developed but simply terminates the style. Make a transection through the three ovaries, and examine with a low power adding a little potash lye. The transection shows us for each ovary a single cavity, Fig. 104. Apparently it is a single fruit-leaf or carpel which forms each ovary. The carpellary-leaf is folded together inwardly and its edges grown fast to each other forming the "ventral seam," so called, which we find in the middle of the ovary on the side which faces the centre of the flower. An ovary formed of one carpellary-leaf is monocarpous, but when several such ovaries are united in one, as in this case, the flower is said to be polycarpous. The ovaries are in this example free to their base where they are inserted on the receptacle and are called superior. The whole female generative apparatus, whether it consists of one or more pistils, is designated the gynecium.

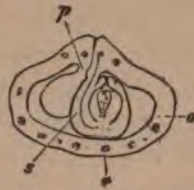


FIG. 104. *Delphinium ajacis*. Transection of ovary. *o*, ovary wall; *v*, vascular bundle; *p*, placenta; *s*, embryo seed. $\times 18$.

Our transection clearly shows the furrow on the ventral side, and by the use of a higher magnification we can easily trace the epidermis at the outside at this place through the whole thickness of the wall and see that it is continued into the epidermis of the cavity of the ovary. Even stomata are found in this inner epidermis. The ovary walls are penetrated by a number of vascular bundles most of which show on the backside, but some near the edges of the carpellary leaf on the ventral side. The edges of the carpellary leaves are somewhat swollen, and, in the cavity of the fruit receptacle, is formed into a placenta, *p*. From this the ovules, *s*, originate in two series, corresponding to the number of the placentæ. We shall give particular attention to the ovule later on, and to this end lay aside our preparation.

In the blossom of *Butomus umbellatus* are six ovaries. But these ovaries are free only in their upper halves, while they are grown together laterally below and cannot be separated without injury. The pistil is very short and the upper edge of it is the stigma. We must prepare transections both of the free and the united parts of the ovary. In those of the upper part we may easily distinguish the carpellary leaves as in the *Delphinium*, but in the sections from below they cannot be isolated intact laterally from each other. In the *Butomus* we have an intermediate form between the polycarpous and monocarpous flowers, and this will serve us as an example of a compound ovary formed out of more than one carpellary leaf. A marked peculiarity of the *Butomus* is seen in the fact that the ovules do not spring alone from the edges of the carpellary leaves, but rather from their middle and from their whole inner surface. The whole wall of the ovary is beset with them and acts as a parietal placenta. At the point of insertion of each ovule a fine vascular bundle may be seen,

which provides for the ovule. These are branches of larger bundles lying deeper in the tissue.

The carpellary leaves of the *Liliaceæ* are superior. Take for our investigation a tulip, hyacinth, a lily or a *Hemerocallis*. In the tulip the three stigmas rest on the ovary without a style. In the hyacinth the style is short, the stigma small, dark, divided into three parts. In the lily the style is long, the stigma three-parted. In *Hemerocallis* the style is very long with a three-parted, still very small stigma. A transection will show us a compound ovary formed of three closed carpellary leaves grown together. No boundary between the parts either at the side or in the middle is to be recognized here. A continuous epidermis covers the whole organ. Three carpellary leaves form a compound ovary with three cells. Each of the three carpels which form this ovary has two series of ovules, lying along its two edges. The placenta therefore lies in the inner angle of the ovary cell. The placenta is therefore marginal as in *Delphinium*, and since it springs from the angle of the ovary which turns toward the middle it is called "central." A transection of the pistil of *Hemerocallis* shows us a three-cornered style, in which toward the three edges are distributed three vascular bundles. A longitudinal section of the pistil which cuts the stigma will show that the latter is developed into long papillæ on its upper surface. This is the most common appearance of the stigma. But in the *Hemerocallis* we find the cuticle of the papillæ raised up by the pressure of mucilage formed beneath. The cuticle is spirally striped and conformably to this the elevations follow a spiral line. The cuticle will finally be separated from the inner layer and eventually removed from the papillæ. The other *Liliaceæ* might likewise show us a hollow style, but in most flowers it is solid, filled with cells with swollen

side walls, or with those entering from the lateral tissue, between which the pollen tube can easily grow downwards.

The *Primularia* species have a superior ovary. They are dimorphic, that is they show short and long styled ovaries and stamens inserted above and below on the corolla. A median longitudinal section through the ovary shows us that the axis of the flower continues into the cavity of the ovary and expands into the shape of a toadstool. In the middle this expansion rises into the style of the pistil in a papillate form and the whole upper surface is beset with ovules. We have in this case a free central placenta. The walls of the ovary nowhere join this placenta, as is seen by a transection in which these walls appear as a free ring about the central placenta. The point of juncture or suture is not visible in this ring; so, in order to determine the number of carpellary leaves which go to form the ovary, we must refer to the number of the other parts of the flower and to the circumstance that in many *Primulaceæ* the seed capsule opens at the top with five teeth, and thus conclude that there are five. In *Primula* itself the number of teeth with which the capsule opens is indefinite. Instead of the *Primula* we may take species of the *Lysimachia* or *Anagallis* for an investigation, as they have all their ovules on a free central placenta.

We shall now take an inferior ovary—that of the *Epipactis palustris* or some other orchid. The brown ovary lies beneath the other floral parts. We will select for our section a young fruit over which the floral leaves have already begun to be brown. The transection is very instructive. It shows us a simple ovary which bears on the walls at equidistant points three double pairs of placentæ. The placenta divides repeatedly on the edge

and bears a large number of ovules. Upon the outside of the ovary are six projecting ribs, three of them corresponding to the place of insertion of the placentæ within and the other three, especially large ones, alternate with these places. Each rib is furnished with a vascular bundle or with a complexity of them, and besides this a small one at the place of separation of two placentæ. If we were to bestow no thought upon it, this section would seem to agree perfectly with one made from a superior ovary, the ovary would appear to be formed from three carpellary leaves and the pairs of placentæ to be produced on the united edges of two such adjacent leaves, and the three ribs which alternate with the places of insertion of the placentæ would be held to be the midribs of the leaves. But as this is an inferior ovary the case is far less simple. We may suppose either that the inferior ovary is formed from an excavated floral axis terminated above with carpellary leaves and that from the latter the placentæ continue downward into the excavation, or we may suppose that the carpellary leaves are grown to the hollow floral axis; consequently the outer part of the wall of the inferior ovary belongs to the stem, and the inner part to the carpellary leaves. The latter supposition is decidedly to be preferred, but it has no other than a phylogenetic value, that is to say, we represent to ourselves that in the course of time the inferior ovary is thus produced. But in reality in the object itself there is no moment in its developmental history when it anatomically answers to this supposition. We must be content, therefore, to demonstrate that the structure of this inferior ovary is not essentially different from that of a simple, polymerous, superior ovary. By examining a ripe fruit capsule of *Epipactus*, we shall find that, as in most orchids, the wall splits into six longitudinal openings, the segments between remaining united at

the top and bottom of the ovary. Three of them are broad and fertile and three narrow and sterile, the latter corresponding to the median ribs which we saw in the transection of the ovary, forming the intermediate sections. The three fertile segments bear the placentæ.

We shall next undertake to investigate the structure of the ovule and the process of its fertilization in the angiosperms. In order to get a view of the separate parts of the ovule, make a transection of the ovary of *Aconitum*

Napellus or of some other species of this genus. Take a flower, just turning blue, strip off the floral parts and make a section of the three ovaries at once, care being taken that the section is really at right angles with the axis of the ovary. Make a considerable number of sections, so as to be sure to have one which cuts the ovule at the right place. Look them over and select the best section, and in case it is not thin enough a little potash lye will help make it transparent. The im-

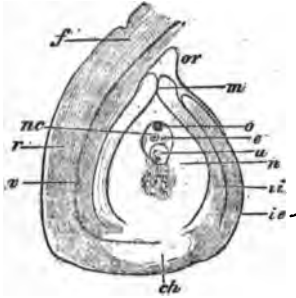


FIG. 105. *Aconitum Napellus*. Longitudinal section of ovule; *f*, funiculus; *r*, raphe; *v*, vascular bundle of funiculus; *ie* and *ii*, outer and inner integument; *n*, nucellus; *ch*, chalaza; *e*, embryo sac; *a*, antipodal cells; *o*, egg; *nc*, nucleus of the embryo sac; *m*, micropyle; *or*, wall of the ovary. $\times 53$.

age will be almost identical with that of the previously examined *Delphinium*. Still the structure of the envelope of the ovule is a little different, and this difference gives it the preference. The ovary is monomerous. The ovules spring from a placenta formed on the infolded edge of the carpellary leaves. They are inserted with a small style or funiculus, *f*, whose free part is quite short, but the rest of it is grown fast to the body of the ovule and forms the so-called raphe, *r*. In the body of the ovule we distin-

guish, first of all, the inner cone-shaped mass of tissue, the ovule nucleus, or nucellus, *n*. This corresponds to the macrospore of the vascular cryptogams. It is surrounded by two integuments: an inner, *ii*, and an outer, *ie*. The inner one is developed all around down to the base. The outer one fails on the side of the funiculus, with which it is laterally connected. Between the upper edges of the inner integument is a free opening or canal, the micropyle, *m*, down to the nucellus. In the funiculus a vascular bundle coming from the placenta may be traced, sometimes, but not always, quite down to the base of the nucellus. At the base of the nucellus is a mass of clear tissue called the base of the ovule, or chalaza, *ch*. In the axis of the nucellus is the large cavity-forming cell of the embryo sac, *e*. At the base of this are some spherical cells, which in the *Aconitum* and in *Ranunculaceæ* generally are strongly developed; they are the so-called antipodal cells, *a*. In specially favorable cases they may be seen to occur in threes. At the apex of the embryo sac a small cell may be made out—but only in an exactly median section—which is the ovum cell, *o*. The whole ovule is anatropic or recurrent because the body of the ovule is not an elongation of the funiculus, but is folded back upon it and partly surrounded by it and grown fast to it, the micropyle being turned towards the base of the funiculus. This form of ovule largely prevails in angiosperms. If we now compare this preparation with that of *Delphinium* we shall find that the structure is almost identical with it, the only difference being that in the *Delphinium* the two integuments of the ovule are united into one.

In order to make a good section of the ovule in the ordinary way between the thumb and finger we must remove it from the ovary. If it is rightly placed between the thumb and finger we may obtain a median view this

way sooner than by any other. But we may with advantage embed the ovule in glycerine jelly or in collodion before cutting. The glycerine jelly must be relatively stiff, that is, contain considerable gelatine. Only alcohol material can be embedded in collodion. Pour the collodion solution in a little box made of writing paper and lay the ovule in it. It must stand in the air till it stiffens so that it will not run, then put it in 60 to 90 % alcohol. Here it will, in the course of a few hours, become of the consistency of gristle and be transparent; cut through the object and the collodion together, and transfer the section without removing the collodion to glycerine or glycerine jelly. If one has got his collodion in the form of tablets he must dissolve it in a solution of equal parts ether and absolute alcohol. In order to make the ovule visible in the embedding medium it may be first stained in an aqueous solution of hæmatoxylin. But the water must be afterwards removed from the object by the use of absolute alcohol before it can be embedded in the collodion.

For the study of the interior of the embryo sac we will take the *Monotropa Hypopitys*, or false beech drops (2), the pale yellow plant common in pine forests. It is such a very favorable object for our purpose that we should spare no pains to obtain it. It blossoms in June and July and must be examined fresh since alcohol turns it dark brown and makes it opaque. It may be kept for a long time in a glass of water. Species of *Pyrola* answer the same purpose, only that the ovules are smaller. A trans-section of the superior ovary shows it to be four-celled. The placentæ are much swollen and bear on their surface small, very numerous, closely compacted ovules. The two halves of the placentæ in each compartment are widely separated by radial lines. In the upper part of the ovary these lines reach the middle and touch each other. We

now see four stout pairs of placentæ fixed to the middle of the division walls which belong to each two neighboring compartments, the pairs being easily separated with the needle. Remove the ovules from the open placentæ with a needle and put them in pure water or a 3% solution of sugar in which the ovules will keep a long time. If we get our material from an old flower in which the stamens

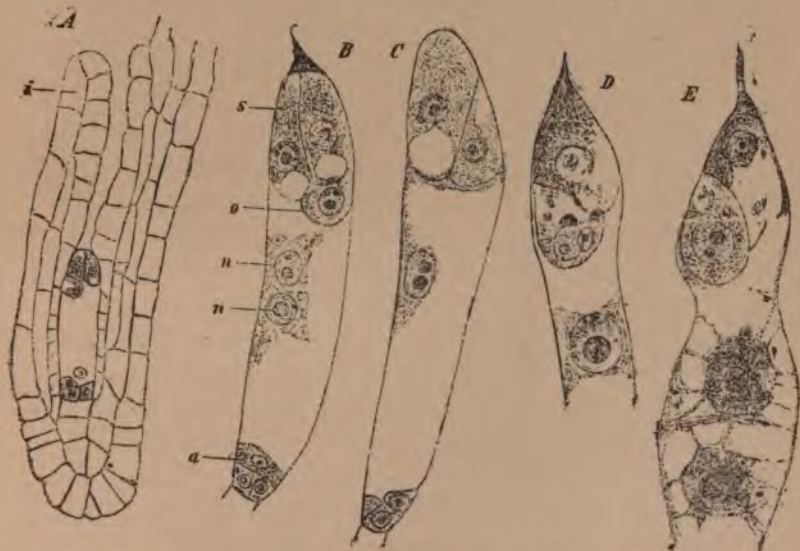


FIG. 106. *Monotropa Hypopitys*. A, a whole ovule; *f*, the funiculus; *i*, the integument; B and C, the whole embryo sac; *s*, synergids; *o*, egg; *n*, nucleus of embryo sac; D and E, the upper part of the embryo sac. In E, is the first division for the formation of the endosperm. A $\times 240$. B to E. $\times 600$.

have already discharged their pollen, we shall find the ovules ripe, but in part already fertilized, and in part not. Between the ovules we shall find many pieces of pollen tubes. The ovule ripe for fertilization is seen as in Fig. 106, A. It is transparent and may be seen in optical sections. It is anatropic and has but one integument, *i*. The whole interior of the ovule is filled with the embryo

sac, the nucellus being suppressed by the growth of the embryo sac. We will assume that the three cells of the apex of the embryo sac are clearly seen. These three cells form the egg apparatus. They are not of equal value. The two upper ones are helpers or synergidæ, Fig. 106, *B*; the lower one is the true egg, *o*. The synergidæ, as may be easily seen, have large vacuoles in their lower part and are filled above with protoplasm and at that point have their nuclei. The egg, on the contrary, lies between the principal mass of the cell plasma and cell nucleus and above the cell cavity. We may not always see both synergidæ, as one may cover up the other, Fig. 106, *C*. At the base of the embryo sac one may see the three antipodal cells. In the interior of the embryo sac, one may find in most cases a cell nucleus, Fig. 106, *A*. Still in other cases there are two nuclei, Fig. 106, *B*, or one cell nucleus with two nucleus bodies, Fig. 106, *C*, and we conclude that in the end the cell nucleus is made from the union of two nuclei. Ovules, whose fertilization has begun, show the fact in the changes which have taken place in the synergidæ. One or both of them appear strangely refractive. A pollen tube may also be seen penetrating the embryo sac, or at least, within the micropyle, or a piece of it projecting from the micropyle, torn away in the preparation. But, if the pollen tube has penetrated to the synergidæ, the plasma from it will be thrown in between these cells upon the ovum itself. By careful examination, it will be seen that an ovum which lies near these changed synergidæ has two nuclei: one large, the original nucleus of the ovum, and a much smaller one which is the sperm nucleus from the pollen tube, Fig. 106, *D*. The latter soon increases in size. If one finds the ovule at the moment of copulation between the egg nucleus and the sperm nucleus he will see but one germinal nucleus with two nuclei of un-

like size, Fig. 106, *E*, of which the smaller is the spermatie nucleus. At last the germinal nucleus will have but one nucleolus. While the fertilization of the ovum is going on the strongly refractive substance in the synergidæ cells is lessened, apparently being used up in nourishing the ovum. At the same time with these changes in the egg apparatus, the endosperm has begun to form in the cavity of the embryo sac, by the development of division walls in the sac itself, in this case by direct cell-division; but in other cases, as frequently or more often, the embryo sac nucleus and its derivatives freely divide first, followed later by the formation of division walls between the nuclei. This process, as it commonly takes place, is accompanied by a gradual but not considerable increase in size. When, on the contrary, the embryo sac rapidly grows after the completion of the fertilization of the ovum, it takes place first by nucleus and not by cell division, the cell-building coming later when the embryo sac is nearly full grown. In consequence of the fertilization, the ovum has taken on a delicate cellulose membrane and soon begins to elongate tube-like and after a time penetrates the endosperm body with its apex, where it forms an embryo of a few cells. We have examined the embryo seed only in pure water or in sugar solution. If we would have the nucleus especially distinct, we should examine it in a 2% solution of acetic acid. We thereby fix the nucleus and make it very sharply distinct and also preserve it in that state of self-division in which it was at that moment. Staining media are not recommended since they stain also the integument and thereby hinder the examination of the interior of the nucleus.

Instead of *Monotropa* the orchids (3) would serve us. Fertilization takes place a long time after the discharge of the pollen in the already greatly swollen ovary. Cut

away the ovary and remove an ovule from the placenta with a needle and transfer it to water or a 3 % solution of sugar. As represented in Fig. 107, *m*, we see that the structure of the ovule is much like that of *Monotropa*, except that there are two integuments, and an air cavity in the vicinity of the chalaza. The air cavity hinders the

observation since it is filled with air which finally works up between the integument. It may be taken out with the air pump or perhaps by a light pressure upon the cover-glass. In the orchids the nucellus is quite suppressed by the embryo sac. The egg apparatus, *os*, is built like that of *Monotropa*, only that the ovum is less deeply inserted. The antipodal cells are not to be seen, but in their place a strongly refractive substance in which are nuclei very difficult to make out.

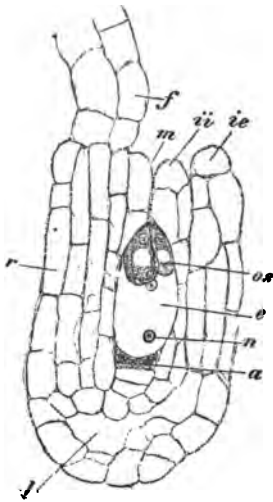


FIG. 107. *Orchis pallens*. Ovule ripe for fertilization. *os*, egg apparatus; *ii*, *ie*, inner and outer integuments; *l*, air cavity. The rest of the letters are the same as in the earlier figure.

It is easier to trace the pollen tube to the synergidæ than in the *Monotropa*; the changes which take place in the synergidæ are quite the same. We also find again the two nuclei in the fertilized ovum. Endosperms are not usually formed.

In lack of *Monotropa* and orchids the transparent ovules of the *Gesneriaceæ* (4) are to be commended and before all others the large-flowered *Gloxinia hybrida*. The ovule with an integument is so far transparent that the egg apparatus is distinctly visible. It shows the two syn-

ergidæ and ovum, in this case fork-shaped. Sometimes two ova appear. The embryo sac is swollen above, but is suddenly narrowed below. The antipodal cells are not made out with certainty.

But one of the most favorable plants for the study of fertilization is the *Torenia asiatica* (5). It is cultivated in almost all gardens and bears flowers the year around. It is distinguished by having the embryo sac protrude through the micropyle of the ovule, and so the egg apparatus comes into view, covered only by the wall of the embryo sac. The ovary is two-celled, the placenta central, with many ovules. Remove some of the ovules and examine in 3 % sugar solution. The ovules are anatropic or more rightly somewhat campylotropic for the embryo sac, and its integuments are somewhat bent in their upper part, Fig. 108, *A*. The funiculus, *f*, is somewhat large and the single integument stout. The embryo sac, *e*, shows its upper end protruded through the micropyle, smaller and pointed and lying against the funiculus. By means of potash lye, at the outset of its action, the embryo sac may be traced downwards into the ovule, where it may be seen to lie next the integument, very slender, somewhat spindle-shaped, *e**, and at the base again narrowed. Our preparation in sugar water shows the three cells of the egg apparatus in the apex of the embryo sac. According to the position of the ovule, will both synergidæ or one be shown, as in *B* or *C*; in the latter case one covers the other.

At the apex of each synergida a strongly-refractive, homogeneous cap, clearly defined against the fine-grained posterior part, may be easily seen. It is called the fili-form apparatus. Chloriodide of zinc shows by the violet reaction that this cap consists of cellulose. The rest of the substance of these cells and of the ovum is colored

yellow-brown by this reagent. By careful examination, we find that the embryo sac membrane is opened over the synergidæ cap, *B*, *C*. The cap, therefore, forms the closing apparatus for this opening of the sac membrane. It may be remarked in passing that they are widely dis-

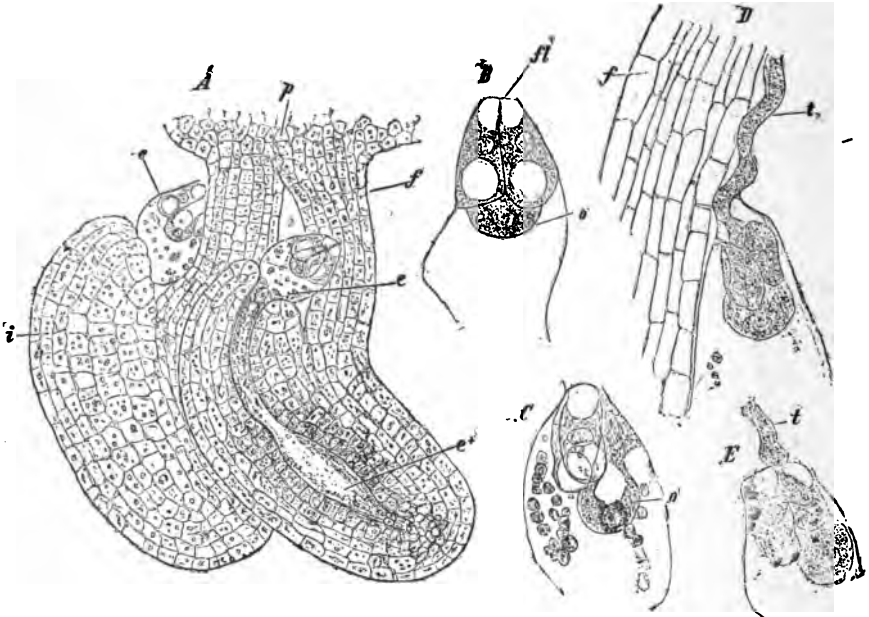


FIG. 108. *Torenia asiatica*. *A*, two ovules on the placenta *p*; *e*, the free apex of the embryo sac; *e**, the lower widened part of the same in the interior of the ovule; *f*, funiculus; *i*, integument. $\times 240$. *B* and *C*, free apices of embryo sac before fertilization; *st*, synergidæ caps, filament apparatus; *o*, egg; *D* and *E*, during fertilization; *D*, with a part of the funiculus, *f*; *t*, pollen tube. *B* to *E*. $\times 600$.

tributed, particularly in monocotyledonous plants and are often found in them protruding some distance out of the embryo sac. Their striation, which is often observed in these plants, is found to consist of fine pores filled with plasmic contents.

By turning back to our preparation we find the distri-

bution of the contents in the synergidæ and the ovum is the same as in the *Monotropa* and *Orchids*, *B*, *C*. In the synergidæ the nucleus lies in the upper part and the vacuole in the under. This is reversed in the egg.

If we wish to study the process of fertilization in the *Torenia* we must pollinate the flower. Thirty-six hours are required to complete the process, so we must begin our examination a day and a half or two days later. Remove the ovule from the placenta under the simplex with the greatest possible care, taking therewith as much as possible of the pollen tube. It will then be very easy to trace its course to the apex of the embryo sac and down between the synergidæ caps to the egg, *D*, *E*. One sees that the pollen tube from the placenta leads down the funiculus till it reaches the apex of the embryo sac. The latter exercises a direct influence upon the direction of the growth of the pollen tube. For it is supposed that the synergidæ secrete a substance which acts as a kind of stimulus on the pollen tubes. On account of the soft nature of the caps they offer little resistance to the discharge of this substance. When they are strongly developed they are found to be perforated with fine canals by which the secretion is discharged. The synergidæ in *Torenia*, as elsewhere, become disorganized after the entrance of the pollen tube, and assume the refractive appearance already referred to. This object is not suitable to the further and concluding process of fertilization.

NOTES.

(1) Goebel, Grundzüge d. Syst., etc., p. 417; Lürssen, Grundz. d. Bot. p. 356; Med. Pharm. Bot. Bd. II, 244; Prantl, Lehrb. d. Bot., IV Aufl., p. 195.

(2) Strasburger, Befr. u. Zellth. pp. 34 u. 35.

(3) The same work, p. 55.

(4) " " " " 54.

(5) " " " " 52.

LESSON XXX.

STRUCTURE OF THE SEEDS OF THE ANGIOSPERMS.

WE shall now study the structure of the ripe seeds and give especial attention to the germ which they bear. Take the *Capsella bursa pastoris*, a plant often used for embryological studies (1). Its seed is relatively quite small, but all the better on that account for an investigation into the history of its development. First, make a longi-

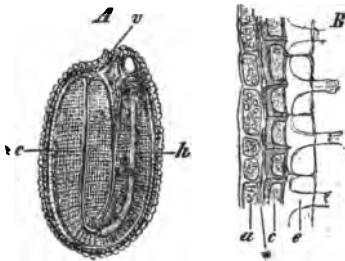


FIG. 109. *A*, longitudinal section of ripe seed of *Capsella bursa pastoris*; *h*, hypocotyledon; *c*, cotyledon; *v*, vascular bundle of funiculus. $\times 26$. *B*, longitudinal section of the seed coat after soaking in water; *e*, swollen epidermis; *c*, brown much thickened layer; *, compressed layer; *a*, aleuron layer. $\times 240$.

tudinal median section, so that we may know how the object looks whose development we are to study. This may be done without too great difficulty with a fresh seed between the fingers, but may perhaps be more successfully accomplished by holding it between two flat pieces of cork, or by gumming it between two pieces of soft linden or poplar wood and

then cutting through the wood and seed together when the gum is dry; or, finally, the seed may be embedded in the end of an elder stem which has been hollowed out, in a drop of gum, and when the gum has dried cut the desired section.

The section should be examined in glycerine, since water swells the germ and so pushes it out of the seed coat. The germ, Fig. 109, *A*, fills the whole body of the seed.

It is bent double in the middle, so that the cotyledon, *c*, lies next to the hypocotyledon, *h*. This manner of placing the embryo is characteristic of the suborder *Notorhizææ* among the *Crucifereæ* and may be represented by the figure 110. If the section is strictly median and sufficiently delicate as in the figure, one may see the small vegetative cone of the stem at the base between the cotyledons, and also at the radicular end of the hypocotyledon a root-cap but a few cell layers thick. There is no endosperm, but the germ is surrounded immediately by the seed-shell or testa. If we now take a stronger magnification we shall be able to demonstrate that this seed coat is composed of three layers of cells, Fig. 109, *B*, an inner layer of cells, *a*, of relatively thin, colorless walls and granular contents. Testing by a solution of iodine, we find the grains colored a yellow-brown and therefore must be aleuron or proteid matter. Next to this layer is one, *c*, with the cell walls a deep brown and much thickened towards the inside. The outer cell layer appears like a colorless, homogeneous membrane in concentrated glycerine, its cells being much flattened and thickened till the cell cavity quite disappears; between the inner and second layer of cells is a layer of flat, compressed cells which appears to be a simple membrane. If we look at the seed from the outside we shall easily recognize the outline of the polygonal cells which make up the outer layer. These cells are separated in part in their inner portion by air-filled, intercellular spaces. In the middle of each cell is a part which, not distinctly marked in itself, is strongly refractive. The walls of the next inner layer are brown, much thickened, the cells themselves only a little smaller than those of the outer layer. Considerably smaller and much less thickened are the cells of the third layer which contains the gluten grains.

If we now admit a little water from the edge of the cover-glass, the cells of the outer layer will rapidly swell and put out a strongly refractive little column from the middle of each. The whole cell cavity disappears being filled with the thickening layer of the wall. The innermost thickening layer forms the remarkable column which protrudes from the surface. The intercellular spaces have disappeared. The swelling walls are distinctly laminated. By further addition of water, the cuticle of the cell will be detached and the outer thickening layer will dissolve in the water in invisible mucilage. The column remains indicating the position of the middle of each cell, Fig. 109, *B, e*. It has not inconsiderably increased in size and one may see the remainder of the dissolved thickening layer attached to its apex. The middle lamella which remains, not having swollen, is not so high as the column. All this is seen in the illustration, Fig. 109, which represents a section of the testa after it has been subjected to the action of water. All this can be seen taking place more rapidly if the section be first examined in alcohol and then water added.

The thickening layer of the outer cells of many seeds shows this peculiarity of forming a mucilage in water. It serves the double purpose often of gluing the seed to foreign objects which carry it far from the parent plant, and also furnishes a viscous reservoir of water upon the outside of the seed.

Since it is difficult to make sections of perfectly ripe seeds it is better when we only wish to study the position and the structure of the embryo, to take seeds which are yet soft and unripe, and use the wholly ripened seeds only in a study of the testa. We will now go back to the younger stage and put the whole embryo seed in potash lye. We shall get these unripe seeds best by splitting

the little pod in halves and then with a scalpel taking them out of the halves. Till the seed is almost ripe it may be thus made sufficiently transparent to enable us to see the exact position of the embryo. The embryo becomes a beautiful green in the potash which shows that the starch grains swell and the chlorophyll grains become thereby visible. We see thereby that the embryo is shorter the younger the seed we examine, and especially is this true of the cotyledons. It is withdrawn more and more from the under half of the cavity of the embryo sac which is bent upwards. Seed buds from pods which without the style measure not more than half a centimeter in length show the embryo as a small heart-shaped body. The two projecting separating processes are the beginnings of the cotyledons.

As we follow the successive stages of the development of the seed we shall see that the endosperm is formed only at the two ends of the embryo sac and principally at the chalaza end as a green tissue body. Only in the nearly ripened seed will this be reached and supplanted by the cotyledons. We also shall see that the testa is formed, the outer layer from the outer integument of the ovule, and the inner layer of cells from the inner integument. The latter is early distinguished by its rich cell contents. Between the inner and outer integument is a layer of cells one or two cells thick which gradually is extended and compressed till it finally forms a thin membrane between the second and third layers of the seed coat.

In order to study the egg-apparatus of the ovule at the time of its fertilization, we must use alcohol material which we have previously sufficiently clarified with potash lye. We see the two synergidæ and the oöspore in the egg apparatus but the antipodal cells are very difficult to make out. The structure of the ovule is easily traced in the fresh

material examined in water, or when made more transparent by the addition of a little potash. The ovule is campylotropic; that is, its nucellus and embryo sac are bent double as we have already seen in the older seeds. The outer integument consists of two layers of cells, the inner in its upper part of two and further along of three. In this stage of development the nucellus is already supplanted, so that the embryo sac rests directly upon the inner integument. The funiculus is pretty long and is furnished with a vascular bundle which however ends at the chalaza and is still to be seen in the ripe seed, Fig. 109, *A, v*. The next stage in the development is very interesting. It may be studied without the aid of potash lye. We observe that the fertilized oöspore has grown out into a filamentous protogerm about six cells long. The upper part of this, that is the cell farthest removed from the micropyle, rounds out into an embryosphere, while the lowermost cells of the embryo-carriers, or suspensors, puff up bladder-like, supplant the whole nucellus tissue quite to the integument and form the bladder which we find in this place already complete.

In such preparations we are able to see that the embryo spherule is separated from the suspensor by a division wall, and is then parted longitudinally by an horizontal wall and again by another like wall at right angles to the first and then finally into eight parts by a transverse division wall. The embryo spherule increases in size and, in the number of its cells, becomes a little compressed at its anterior end from which the cotyledons grow. Between the bases of these is the vegetative cone of the stem.

For a study of the germ of the monocotyledons, we will take the common water plantain *Alisma plantago* (2). First of all, we will make ourselves familiar with the full grown form. The blossom contains several monomerous

ovaries. It is polycarpal. From each blossom several seeds are produced pressed closely together forming a compound fruit of triangular outline. Each seed is much compressed, thicker above, a reverse ovate in form with a median furrow on the back. On the inside edge of each seed about half-way up projects a short filiform process corresponding to the withered pistil. For our section we



FIG. 110. *Alisma plantago*. Median longitudinal section of ripe fruit; *ep*, epicarp (epidermis); *m*, mesocarp; *en*, endocarp of the fruit wall, pericarp; *v*, vascular bundle; *v**, end of same; *st*, dead pistil; *t*, style; *f*, funiculus of seed with vascular bundle, *fv*; *mp*, micropyle; *ch*, chalazal end; *ts*, seed coat, testa; *hp*, hypocotyledon; *fl*, primary leaf; *cl*, cotyledon. $\times 28$.

will take a seed not quite ripe and between the two halves of a cork stopper make our section, the seed coat being too hard to do it conveniently while holding it with the fingers. We will also make some transections in the same way. The longitudinal sections should be examined in water to which some potash lye has been added. For the transections pure water will do.

To remove the air from the former section for the examination of the seed coat it may be put for a short time in alcohol or under the air pump. Lay some of these sections also in carbolic acid and so obtain views of the structure which, in important respects, supplements those obtained from the other. The longitudinal section is illustrated in Fig. 110. We have first the relatively thick pericarp covered with the epidermis, *ep*. The latter is a

pretty clearly distinct part of the pericarp and may be called the epicarp. Next to this is the mesocarp, *m*, parenchymatous tissue of very nearly isodiametric cells filled with air. The endocarp, *en*, consists of several layers of elongated sclerenchyma elements. An exactly median section will open a mucilage passage on the back side of the pericarp. This can be best seen in the unripe seed, since in the ripe it is almost empty of contents and is scarcely distinguishable from the surrounding tissue. A section, not exactly median, will lay bare a vascular bundle, resting on the endocarp, and, entering on the back side, *v*, passing over and down to the front of the fruit to *v**. At the point where the pistil, *st*, is inserted, the pericarp projects in a sharp edge formed of elongated cells. In favorable cases, an air-filled passage, *t*, ending at the pistil and extending nearly to the seed cavity, may be made out. It is the way by which the pollen tube reaches the ovule. Since the ovule has its micropyle turned towards the back side of the ovary, the pollen tube must pass around the funiculus after entering the cavity of the ovary.

The layers of the pericarp and the furrow in the back side of the fruit are more distinctly seen in the transection, than in the longitudinal section. As is seen in the latter section the seed very nearly fills the cavity of the ovary and is held in a central position at the base of this cavity by a pretty long, bent funiculus, *f*. The vascular bundle enters by the funiculus. The seed is campylotropic and is completely filled by the embryo. The testa, *ts*, is a thin membrane consisting of two distinct cell layers, but a third may be sometimes seen after treatment with potash lye. The micropyle projects sharply from the seed. The root end of the germ lies directly within the micropyle. On the left and within about half-way up the seed is a small

indentation in the embryo. Here lies the vegetative cone of the stem and from it arises the beginning of the first leaf, *fl*, which completely fills the little cavity. The hypocotyledon lies between the vegetative cone and the root, is covered with an epidermis, and has three layers of rind cells regularly arranged, and a central cord of elongated cells which extends from the apex of the root towards the vegetative cone. These rind layers have but one common initial layer at the apex. The dermatogen runs over this from which the two root-caps seem to be derived. The hypocotyledon continues into the cotyledon which may be seen in the germinal cavity bent double, gradually diminishing in size towards the end, which terminates at the chalaza end of the seed. The cotyledon also consists of concentrically-arranged layers of cells wrapped around a central cord of elongated cells. This cord bends under the vegetative cone and continues into the hypocotyledon. The cell layers of the rind also pass from the latter over into the cotyledon. The cell layers of the rind diminish upwards towards the attenuated point of the cotyledon from three to one, the central cord ending some distance below the apex of the cotyledon. There is no trace of an endosperm in the ripe seed. The embryo is closely packed with starch in all its cells.

A transection gives nothing new, but shows the concentric arrangement of the cells very clearly, and affords a better view of the structure of the testa than the longitudinal section.

These two illustrations of the method of forming the embryo in the angiosperms are typical forms of the dicotyledons and monocotyledons, but are very far from being typical of all the cases which have been observed. For there are dicotyledons which possess but one germinal

leaf (*Carum bulbocastanum*, *Ranunculus ficaria*), and monocotyledons where the germinal leaf is produced laterally from the adjacent terminal vegetative cone of the stem, *Dioscoraceæ*, *Commelyneæ* (3).

NOTES.

(1) See Hanstein, Bot. Abhandl. Bd. I, Heft 1, p. 5; Westermaier, Flora 1876, p. 483; Famintzin, Mém. de l'Acad. imp. d. sc. d. St. Petersb., VII sér., T. XXVI, N. 10; Kny, bot. Wandtafeln, Heft I, p. 20. Eine Zusammenstellung aller embryologischen Arbeiten in Goebel, Vergl. Entwicklungsgeschichte, in Schenk's Handb. d. Bot. Bd. III, p. 165, ff.

(2) Hanstein, quoted above, p. 33; Famintzin, quoted above, p. 4.

(3) The literature in Goebel, l. c. p. 169, ff.

LESSON XXXI.

THE FRUIT OF THE ANGIOSPERMS.

FOR the study of a fruit capsule of more complicated structure than that of the orchid already examined we will take a ripe plum, *Prunus domestica*. On the surface is a delicate covering of down. The epidermis of the plum is composed of cells arranged in groups which betray their origin in a common mother-cell. They contain a rose-red cell-sap. A delicate transection will show that the cells under the epidermis for several layers deep rapidly increase in size and then remain of like size beyond. They are rounded up towards each other and yet form only small intercellular spaces. They contain a few very small yellowish-green chlorophyll grains, a thin wall layer of protoplasm, and a nucleus, elsewhere colorless cell-sap. This tissue which is penetrated with numerous vascular bundles is composed, nearer the stone, of smaller and radially elongated cells. The stone itself cannot be cut with a razor without danger of breaking the blade. If such an instrument is to be used a surface must be carefully prepared for cutting, with a pocket knife. The cell walls will be found to be much thickened and lignified and penetrated with delicate, branched canals. A study of the development of the fruit will show that the whole of the tissue of the plum, including the stone, takes its origin from the walls of the ovary, the epidermis of the plum from that of the ovary, the pulp from the mesocarp, the stone from the inner tissue, the endocarp. Within the stone is the seed which consists of the germ, the delicate seed membrane

and the endosperm. A transection shows us the two flat cotyledons, and a median longitudinal section will show at the base between the cotyledons the stem of the germ with its root end at the pointed micropyle end of the seed, and the plumule between the cotyledons at their base. The embryo finally supplants the whole original seed tissue quite to the thin testa, on which laterally from the micropyle the funiculus projects. A delicate transection shows the testa to be composed of a compressed cell-layer beset without by a few or several rounded cells. Between the testa and the cotyledons is an endosperm either wholly suppressed or reduced to a layer of cells. The rounded cells scattered over the surface of the testa are epidermal cells of the testa which have become thickened while their neighbors have remained unthickened and have been compressed. The testa arises from the one integument of the ovule. Two ovules occur in the ovary, but one only is developed.

The observations made upon the plum will serve equally well for the cherry with unimportant differences.

We will next examine the structure of the apple. While the plum and cherry form their superior ovary from a single carpel, the inferior five-celled ovary of the apple is formed from a union of five carpels. As in the nearly related form, the rose, the five-celled ovary may be supposed to be an excavated stem, a so-called hypanthium. To designate the apple as well as the hawthorn-berry a pseudo fruit is in all respects incorrect, since it differs in nothing from the inferior ovaries of many other plants. The apple is crowned at its apex with five more or less perfectly dead sepals and the dried up parts of the flower. A superficial section shows that the epidermis of the apple is formed of relatively small polygonal cells by the arrangement of which it is possible to follow the successive

steps of their development. The walls of the cells are considerably thickened, their cell-sap either colorless or rose-red. The surface is covered with a finely granular waxy substance. The minute elevations of the surfaces which are easily seen with the magnifying glass are occupied in the middle with a stoma. The tissue under these stomata is often dead; and, the surface cracking, the wound is finally closed with a growth of cork. A thin transection will show us that the epidermis is much thickened on the outside and that below this the cells for several layers deep are radially elongated, with thickened walls, which gradually become larger and thinner walled within, and contain chlorophyll. There is therefore no sharp demarcation between epicarp and mesocarp. The chlorophyll grains are closely packed with starch. Their color disappears and they diminish in number towards the interior of the apple. At a certain depth the large, bladder-like cells of the mesocarp have only a wall lining of protoplasm and a nucleus, besides the cell-sap, the intercellular spaces being filled with air.

The five seed chambers are covered with a smooth, hard membrane, the endocarp, which corresponds to the stone of the plum. It consists of several layers of sclerenchyma fibres which are irregularly bevelled, often bent and run into different layers. The five compartments often separate from each other in the middle, forming a central cavity into which they most generally open. At the bottom of each chamber are two ovules, one or both or generally neither of which develop into seeds. The seed is nearly filled with the germ which has the same structure as that of the plum or cherry, the testa being much thicker. A transection of the epidermis shows it to consist of an outer layer of colorless and an inner layer of brown cells, the former being capable of

much swelling, the latter not. If a section be put in water, the former layer soon greatly increases in thickness, and the cells of the cuticle expand and arch out papillately. This is what makes the moist seed slippery. The tissue immediately beneath this consists of polygonal cells rounded at the corners, much thickened and brown. Below this, follows a layer about a third as thick, formed of tangentially-elongated, brown, somewhat thinner cells. These border on the bright, white, thick membrane. The latter arises from the strongly-thickened, outer wall of the centre nucellus layer, the whole of the rest of the testa from the outer integument of the ovule. The inner integument is suppressed earlier. The nucellar cells which we have reckoned in the testa are mostly compressed, as are also the remaining cells of the nucellus. On these flattened cells follows a thin layer of endosperm which is in some places quite supplanted, but elsewhere covers the embryo. The endosperm cells are closely packed with gluten grains. Successive superficial cells show that the epidermis consists of relatively few elongated cells whose inner thickening layer is porous. The next following tissue consists of elongated cells with diagonally arranged dots. The tangentially-elongated, inner elements of the testa are at right angles with these.

A transection of a ripe orange, *Citrus vulgaris*(1), shows us compartments varying from six to twelve, laterally separated by their partition walls and filled with orange-red pulp. The partitions meet in a central tissue-column. We may consider the outer shell or rind the epicarp, the pulp, the mesocarp, the central column and partition walls the endocarp. A delicate transection of the outer rind shows a small-celled epidermis followed by tissue of gradually larger cells. The epidermis and the next following tissue have orange-yellow chromatophores. Then inter-

cellular spaces filled with air follow which gradually become so large as to give the tissue the character of loose sponge-parenchyma. The cells are tangentially elongated. The shell is permeated with vascular bundles which the trans-section usually lays bare lengthwise and which branch outward towards the surface. In the epidermis we see with the naked eye the large receptacles of essential oil, which are of the same structure as those of *Ruta* and are lined with a layer of delicate cells. These oil-cavities appear like dark spots in the fruit. A superficial section shows the cells overlying these receptacles to be lacking in the orange-red chromatophores and to contain in their place large globules. A deeper cut shows the structure of the various oil-holders, and the vascular bundles between them. Still deeper sections bring us to the sponge-like tissue formed from cells elongated into tubes. In the tissue next to the segments the cells of the rind are longer, fibrous, and in part more thickened and furnished with diagonally placed pits. So also the partitions between the segments are built of sponge-like elements on the inside and of elongated thickened cells without. The former easily separate from their connection, the latter adhere pretty well together.

In separating the segments in the usual way the sponge tissue parts while the fibrous tissue remains as a soft white covering to the pulp. This membrane may be used directly for examination. It will be found to consist of unthickened and thickened fibrous cells, the latter pitted. The pulp consists of club-shaped tubes which arise on the outside of the compartment. They are inserted with a slender basis, and pressed together fill the segment. The larger they are and the deeper they extend into the segment the more do they take on a radial arrangement at right angles to the longer axis of the segment. Each of

these club-shaped elements shows that it is surrounded by a layer of connected elongated film-like cells similar to those which we found in the partition wall between the segments. They are much thickened and furnished with diagonally-placed pits. The inside of these club-shaped tubes is filled with large, polygonal, thin-walled cells filled with sap, in whose interior are visible slender, spindle-shaped, orange-red chromatophores. The central column where it joins the partition walls consists of the same sponge-parenchyma which forms the inner part of the "peel" of the orange. In the pulp is embedded an indefinite number of seeds. They occupy the inner edge of the segment, their place of insertion being turned inward. By removing the segment the seed is separated from the placenta. In most cases a part of the tissue of the central column together with the placenta remains adhering to the inner edge of the segment.

We will make a study of the development of the fruit of the orange tree, with reference only, however, to learning the more important phases of its history. A transection of an ovary which has but just shed its blossom shows already a pretty thick envelope, while the segments are relatively small, the central column stout and the oil receptacles already developed in the rind. The ovules are inserted in two rows in the inner angle of the segments with their longer axis extended outward. The segments are enveloped with an epidermis, on which border two or three other closely compacted layers of tissue, while further in the tissue contains air-filled spaces. From the outer surface of each segment already small knobs project inwardly. The inner epidermis and the next following cell layers partake in the formation of these knobs. A transection of a small fruit not more than 5 mm. in diameter, will show us in place of these

knobs, cylindrical, small-celled protuberances, which reach different depths in the segment and begin to penetrate between the embryo seeds. Their epidermis continues into that of the segments, while their inner cells pass over into the hypodermal tissue which surrounds the segments. These protuberances are still found in earlier stages of the development of the fruit, the cells of their surface being papillaceous. The older the young fruit is which we examine the longer will be the tubes which form the pulp of the segments. The segments themselves remain still very small in comparison with the thickness of the growing rind, in the periphery of which are the increasing oil receptacles. The pulp tubes begin further on to assume a club shape in their upper part and to cover themselves with epidermis lengthwise, while their inner cells remain isodiametric by repeated transverse divisions. These cells are filled with yellowish contents. A considerable expansion of the epidermis which covers the segments takes place and also of the layers which lie next to that and which were previously distinguished by lack of intercellular spaces. This all takes place in a young orange not over 15 or 20 mm. in diameter and essentially explains the method of development, for the pulp tubes are not further differentiated than is needful to attain the condition seen in the ripe fruit. But from the epidermis of the segments there arises the fibrous layer which covers the pulp tubes. The loose tissue of the central column and that of the principal rind furnish the sponge parenchyma. In the periphery of the rind the oil cavities may be found in every stage of development, and the layers which now contain chlorophyll are those which at a later period contain the orange-red chromatophores.

A transection of an ovary in blossom treated with potash

shows us easily an ovule in median longitudinal section (2). The ovules are anatropic. We easily make out two thick integuments, a nucellus, and in an exactly median section a small embryosac. Four weeks will elapse between the pollination and fertilization of the orange. There is difficulty in studying the fertilizing process, but if we take an ovule from a young fruit about 20 mm. thick we can easily make a longitudinal section between the thumb and finger and find in the apex of the embryo sac the minute germ. The nucellus is excavated and the course the pollen tubes take to come to the embryo sac is marked by small cells rich in contents. The inner integument is recognized by the brown color of its inner cell layer and the smaller size of its elements. The outer integument is considerably thicker than the inner. On the latter the epidermis begins to fill itself with fine grained contents on the inside and to thicken on the outside. If the ovule has reached a height of 3-5mm. a very peculiar appearance is to be seen. In the immediate neighborhood of the apex of the embryo sac a protuberance arises which is visible in the tissue of the surrounding nucellus. It will produce in this genus as in a number of other angiosperms an adventive germ along with the fertile ovum. A median longitudinal section through the next older seed-embryo will show us, in different stages of development, roundish germ buds projecting into the embryo sac clustered especially in the anterior end. Soon the endosperm begins to develop and in the next older stage we find the embryo sac quite filled with it. In the latter the embryo germ soon begins to develop the cotyledons which assume the form typical of the dicotyledonous plants. The nucellus is repressed quite to the outer cell layer of the embryo sac. The epidermal cells on the outer integument have been considerably elongated, and much thickened without. The rest of the tissue of the

outer and inner integument has undergone no essential change. The germs soon begin to interfere with each other's development, one or the other getting the upper hand after the endosperm is suppressed, and filling out the embryo sac. A longitudinal section of a ripe seed will show us one or more germs pressed upon each other and with these perhaps several which have had their development arrested. The polyembryonic nature of the orange seed does not rest on the existence in the embryo sac of several ova capable of fertilization but on the growth of adventive germs. The testa is produced from the two integuments and the outer cell layer of the nucellus, which latter is full of rich cell contents. The boundary between the integuments has disappeared, but the inner layer of the inner is distinguished by its color. The epidermis on the outer integument has attained a considerable height, and by means of a newly-formed, diagonally-pitted thickening layer is also much thickened on its side walls. The outer thickening mass swells in water making the seed slippery and slimy. The inner thickening layer which is produced last increases in volume and forms papillate projections without.

NOTES.

- (1) See also Poulsen, *Botaniska Notiser* utg. of Nordstedt 1877, p. 97. There the literature.
- (2) E. Strasburger, *Jen. Zeitschr. f. Naturw.* Bd. XII, 1878, p. 652.

LESSON XXXII.

SELF-DIVISION OF NUCLEUS AND CELL.

THE best object for studying the process of cell and nucleus division is the already known hairs of *Tradescantia virginica* or of some related species (1). We should take the hair before it is fully grown, and when it is undergoing a lively increase in its cells. Use a bud 5 or 6 mm. high. First open the bud and with a fine forceps remove the anthers from the filaments. Then cut the ovary across below the insertion of the filaments. Under the simplex, separate the filaments at their base from the ovary with a needle, and examine them in a 3% solution of sugar directly on the object slide or in a hanging drop in a moist chamber. They will live for a considerable time under the cover-glass and may be examined with the highest powers. In the hanging drop they may be kept for half a day in a living and developing state. The drop should be made flat and shallow in order not to have the hair settle so far down as to be out of reach of the higher lenses. The resting nucleus appears finely punctate, Fig. 111, *I*, the under cell; but if we examine it with a higher magnification, or if the cell has suffered somewhat from the influence of the surrounding fluid we shall see that the nucleus is not made of isolated but of connected granules bound close together by fine filaments, so that the whole nucleus represents a network enclosed by a delicate wall. Between these filaments are to be distinguished several nucleus bodies of different sizes.

The nucleus is surrounded by a little protoplasm which is connected by threads of the same to the protoplasmic wall-layer. This plasma contains, besides the scarcely distinguishable microsomes, larger more refractive grains which are leucoplasts. When the nucleus is preparing for self-division it increases considerably in size and gradually

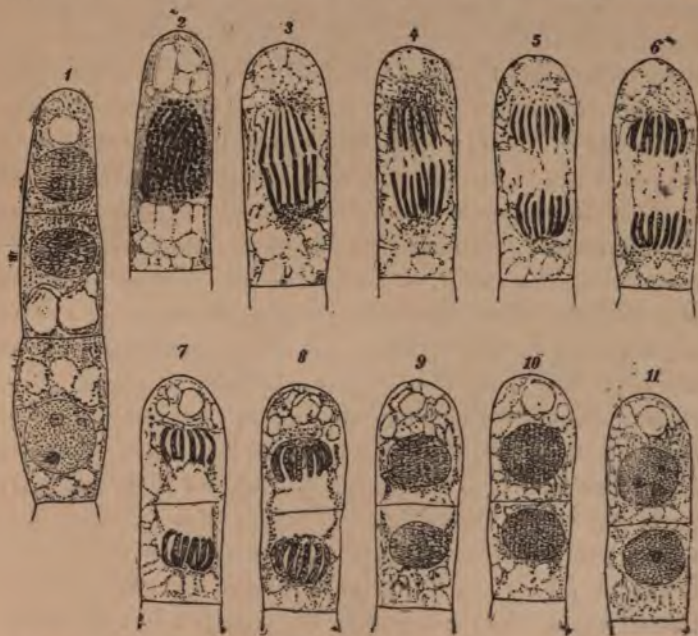


FIG. 111. *Tradescantia virginica*. Process of division of the cells of the hairs on the stamens. 1, A resting nucleus cell below, and in the upper cell a nucleus in the act of dividing; 2, nucleus showing granular diagonal striae; 3-11, successive stages of the process in the same cell: 3, 10 o'clock, 10 minutes; 4, 10.20; 5, 10.25; 6, 10.30; 7, 10.35; 8, 10.40; 9, 10.50; 10, 11.10; 11, 11.30. $\times 540$.

forms large grained threads out of its finely filamentous network. The nucleus now begins to elongate and to arrange its threads in a diagonal direction and nearly parallel to each other, Fig. 111, 2. Finally, the plasma collects in the two poles of the nucleus. One may observe

all of these changes in the same cell but it takes a considerable time. The granules become indistinct in the filaments, and gradually assume a homogeneous aspect. The filaments wind in a definite but not always in a traceable way. By different observations we may conclude that the twist next makes a fold at the equatorial plane of the nucleus and the filaments place themselves parallel to the longer axis of the nucleus. Then the filaments separate at the points where they bend both at the poles and the equator and the figure of the nucleus consists of separate fragments of the filaments which are turned down, hook-shaped at the equator. The next move is obscure, but the stage of development which shows itself next is very distinctly marked. The fragments of the filaments are arranged as shown at 3, in two separate bundles, straight, of nearly the same length, the ends of the segments touching each other at the equator. If these daughter segments are especially long they will be hooked at their polar ends. They are the same number in each bundle. The change from the condition represented in 2 has occupied about one hour. The segments appear homogeneous, but a high power shows the surface to be somewhat beaded, which betrays a structure consisting of successively formed orbicular pieces. At this moment the separation of the two halves of the nucleus may be expected to take place and it will be accomplished so rapidly that it may be seen direct. The two halves of the nucleus draw apart longitudinally, 4. In five minutes they are withdrawn far from each other, 5. The daughter segments do not all always participate in this movement at once, but often some follow the others. They also bend up at the poles somewhat thicker and become correspondingly shorter, 5. Between the two halves then will appear a bright transparent mass which will be increased by the addition of

the plasma masses which have heretofore been collected about the poles, 5 and 6.

In this clear central mass is seen a fine structure which afterwards may be seen to be differentiated into filaments. About twenty-five or thirty minutes after the beginning of the separation, a series of dark points appear in the equatorial plane of the central mass. In the next moment these points coalesce and form a sharply drawn dark line, the new division line. It consequently arises from minute granules. They are microsomes and form what we shall call a cell-plate. It is equidistant from the two halves of the nucleus in the middle of the clear protoplasmic substance, and from it is developed the new division wall. If the central plasma mass is large enough to fill the whole diameter of the cell, we shall see the division wall immediately connected all around with the lateral wall of the mother-cell. But if it only partly fills the cell it will touch one side of the mother-cell wall and the division wall will begin to form at the point of contact. A movement will be set up within the cell which will bring the plasma mass gradually in contact with the mother-cell wall all around, so as finally to complete the division wall. The plasma mass draws away a little from the already formed portion of the division wall and by an adjustment of itself completes what is lacking in that wall, 7-9. During this process the daughter segments bend their equatorial ends inward towards the interior of the nucleus, 7, 8. The ends finally come in contact and coalesce. They immediately begin to assume a finely granular appearance, and with a high magnification they seem to be a very thin filament bent in a zigzag form, 9, and the upper cell in 1. The twist of these filaments is gradually elongated, numerous loops are formed, and they finally anastomose with each other and come to the condi-

tion which marks the end of our observation, *10* and *11*. At the same time the daughter-cell increases in size and it is not improbable that it is nourished at the expense of the surrounding cytoplasm. The newly-formed division wall is slowly nourished by that. About one and a half hours elapse between the beginning of the separation and the completion of the daughter nucleus, and by this time nucleoli are visible in the nucleus, *11*.

Treating with reagents gives in general with this object not very satisfactory results. It is best fixed with 1% acetic acid in order to stain with acetate of methyl green. By this means we are able to show that the central clear mass of protoplasm consists of filaments which connect the two daughter nuclei. We designate them connecting filaments. The central ones are straight, those towards the circumference of the complex being more and more bent. The granules which form the cell plate are found to be equatorial enlargements of the single connecting filaments.

In order quickly to fix any of the various stages in the process of cell or nucleus division we will take the pollen mother-cell in a monocotyledon. The *Liliaceæ*, as *Fritillaria*, *Lilium*, *Alstroemeria* which have particularly large pollen mother-cells and nucleus, are recommended. For *Fritillaria persica* which we shall use here, almost any species of the lily or amaryllis may be substituted. It will be found especially advantageous to select a species which unites in a single plant every degree of development in the floral buds, so that we may have exactly that condition which we need for our examination. We will select a young bud of the *Fritillaria persica*, and taking an anther lay it in a drop of acetate of methyl-green or gentian-violet. Put on a cover-glass and then press upon it with some flat object till the compartments of the anther are

pressed flat and emptied of their contents. The acetic acid will fix the cell contents and the stain will color them and we can then determine whether we have a resting nucleus or one in the process of division. If the pollen



FIG. 112. *Fritillaria persica*. Division of the pollen mother-cell. *a*, ball-shaped form of the nucleus; *b*, the segments undergoing longitudinal division; *c*, the nucleus spindle in profile; *d*, the same seen from the poles; *e*, parting of the nucleus plate; *f*, separating of the daughter segments; *g*, formation of the daughter balls and the cell-plate; *h*, course of the filaments in the daughter nuclei; *i*, their longitudinal elongation and the formation of loops; *k*, nucleus spindle seen at the right in profile and at the left from the pole; *l*, separation of the daughter segments seen at the left from the pole, at the right in profile; *m*, granddaughter balls, formation of the cell-plates. $\times 800$.

mother-cells are already divided into four daughter-cells, or the young pollen grains are already separated from each other, we must seek for a younger bud. We may rec-

ognize the young pollen grain, or the young pollen mother-cell by the thickness of the colorless cell membrane of the latter. We search till we find a thin-walled compound mother-cell whose nucleus is a ball of fine filaments, and to the wall of which are attached flat nucleoli. The reagent contracts the filamentous ball and separates it from the uncolored nucleus wall, Fig. 112, *a*, and one can see that this wall of the nucleus is a membranous layer of the cell plasma (cytoplasm). We shall call those supplementary nuclei, secondary nucleoli (paranucleoli) because it occupies a peripheral position and also behaves somewhat differently from the common nucleolus. This paranucleolus is characteristic of all pollen and spore mother-cells.

As we have in the fibrillar walls and the paranucleolus a preparatory step to the division of the nucleus, so we may pass step by step to the older flowers. For fixing we may use acetic acid with methyl-green, or acetic or formic acid with gentian-violet, or also picro-nigrosin. Preparations stained with the last or with gentian-violet may be preserved in glycerine without further treatment.

A subsequent characteristic stage is seen in Fig. 112 *b*, where segments of the fibrillæ to the number of twelve lie quite evenly distributed about the wall of the nuclear cavity. They are stained with acetate of methyl-green, the cell cavity remaining colorless. The latter is filled, in case we have a young specimen, with homogeneous nucleus sap. An older stage will show, in the nuclear cavity, a greater or less number of delicate cytoplasm fibres. The paranucleolus is but slightly colored and hangs anywhere, to a wall of the cavity or to a segment. These segments arise from the fibrillæ which we saw form before the ball. The filaments are shortened, thickened, flattened and finally are separated into these segments. In favorable cases we

may see each of these segments split in two lengthwise into daughter segments, Fig. 112, *b*, which form a Y- or an X-shaped figure.

The next succeeding characteristic stage is the formation of a nucleus spindle, Fig. 112, *c*. This shows segments equatorially placed and deeply stained which form the nucleus plate, and fine unstained spindle fibres which converge towards the two poles of the nucleus spindle. The fibres join the segments of the nucleus plate. These segments are shaped like a Y with their two limbs, following the fibres, extending towards the poles. The nucleus plate appears from the poles like Fig. 112, *d*. The number of the segments in the plate in this species is mostly twelve. They correspond to the previously observed pairs of segments lying on the wall of the nucleus. The nucleus wall has been dissolved, the surrounding cytoplasm has permeated the nuclear cavity and produced the spindle fibres. Each segment of the nucleus plate is consequently a pair of daughter segments the foot of the Y being formed by the coalescence of the two adjacent ends of the segments caused by the action of the reagent, while the limbs of the letter are formed by the two separated parts of the daughter segments. This completes the preparatory stages of the division of the nucleus.

Now the separation and arrangement of the segments, the intermediate stages in the division of the nucleus begin. This process consists of the separation of the two sister segments of each pair, while at the same time they turn their curvature towards the poles, Fig. 112, *e*. This stage is seldom seen in the preparation since this part of the process is quickly run through. But perhaps the further movements of the dissolution of the sister segments belong to the retrogressive phases of the parting, the anaphases. We see such a condition in Fig. 112, *f*. The

daughter-segments follow the spindle fibres back quite to their polar terminations, where their ends commingle and form the daughter fibrillar balls, Fig. 112, *g*. All these conditions we often find together in the contents of one anther.

While the daughter segments collect at the poles the spindle fibres remain intact as connecting threads between them, Fig. 112, *f*, *g*. Their number increases by the intercalation of new ones till finally they form a barrel-shaped body. They are soon distinguishable only at the equatorial plane, where by thickening they form a series of granules which represent the cell-plate, Fig. 112, *g*. The cell-plate extends across the whole diameter of the cell, its elements commingle and form a division wall which divides the mother-cell into two daughter-cells. In the daughter nucleus there is formed a ball of delicate fibres which run parallel to the original direction of the daughter segments.

Further preparations show us that the fibrillæ in the nuclei of the daughter-cells again become thicker, Fig. 112, *h*. They elongate their twist and deviate from the example in the first nucleus by gradually placing themselves at right angles to their original direction and forming loops at the equator, Fig. 112, *i*. The segments are interrupted at the poles and equator, shorten and withdraw to the equator. Thus the nucleus plate is produced. The spindle fibres are recognized with difficulty, Fig. 112, *k*, at the right. The segments of the nucleus plate are arranged in the form of a wreath, Fig. 112, *k*, left. The two nuclei divide in the same, or in two planes at right angles to each other. Fig. 112, *k*, shows both views. The segments of the nucleus plate divide lengthwise but this is not seen in a preparation fixed in this way. But the daughter segments move asunder and by their less thick-

ness testify to their having been split, Fig. 112, *l*. The further process corresponds to that of the mother-cell. The two cells are thus divided into four granddaughter cells which lie in the same plane or at right angles to each other according to the direction in which the nucleus division has taken place.

For a thorough study of nucleus- and cell-division here represented, this method of fixing is not satisfactory. It is better to put the material in absolute alcohol. Specimens fixed with chromic or picric acid or chromic acid mixture are not so good as those fixed with alcohol. Material laid in absolute alcohol, at least three days, may be cut lengthwise through the anther and the latter put in a solution of safranin in absolute alcohol, after it has been diluted about half with distilled water (2). A drop of the solution may be used on the slide in which to examine the section to find at what stage of development the pollen and the nucleus division has arrived. The section should lie in the safranin from twelve to twenty-four hours and then taken out and washed in absolute alcohol so long as any visible color is given out. Then lay the section in oil of cloves, or better in *origanum* oil, and as soon as it is saturated with it transfer to a solution of dammar in turpentine (dammar dissolved in warm turpentine evaporated to the thickness of syrup) when it will be preserved unchanged. The nucleus alone is colored, the spindle fibres are but feebly marked. Gentian violet will give almost more beautiful results with a like treatment (3).

In order to make the spindle fibres visible, we will lay a number of sections of the alcohol material in a very dilute solution of haematoxylin—a few drops of the haematoxylin in a watch glass full of distilled water. The section should be passed from the alcohol through distilled water

into the staining fluid. This will avoid the presence of a precipitate. Let the section lie several hours in the staining fluid, examining it occasionally with the microscope to test the color. When it is right, mount the section in glycerine. If the section has been excessively colored it may be bleached before putting it in the glycerine, by leaving it for a considerable time in water or by treatment with a solution of alum. The over-colored section may be toned down by treatment with 70% alcohol containing $\frac{1}{4}$ % muriatic acid, and then washed in 70% alcohol or water, which contains a trace of ammonia. But this method requires especial care. Far more beautiful haematoxylin preparations which are equal to the safranin stains are obtained by passing the section stained with haematoxylin through absolute alcohol into oil of cloves or oil of lavender and from this into Canada balsam dissolved in chloroform. The section will need to remain but a short time in the alcohol and in the volatile oil.

One may quickly obtain an instructive preparation from alcohol material by staining with diamant-fuchsin-iodide-green (4). Make a stain of diamant-fuchsin and iodide-green in 50% alcohol. Pour the iodide-green solution in a vessel and slowly add the diamant-fuchsin until the fluid takes on a conspicuous violet color. Put the section of the anther in a drop of the solution for a minute on the slide and then inclining the slide let the fluid run off, and absorb it with blotting paper. Then add a drop of glycerine, arrange the section and put on the cover-glass. The cytoplasm will be colored red, the nucleus blue and the paranucleolus red. For sharpness of outline it stands next to a good preparation stained with safranin and haematoxylin. It may be mounted in Canada balsam or gum-mastic. The former is to be preferred on most accounts

but has the disadvantage, for use with homogeneous immersions that the oils employed dissolve it.*

If gum mastic is to be used the delicate object must be protected from the pressure of the cover-glass. This may be done by drawing some ridges of the gum on the slide where the edges of the cover-glass would come and letting it partly dry immerse the object in the gum between them and lay on the cover-glass. Cement by subsequent application of the dissolved gum, or of gold size. A small drop of wax applied to the slide will serve the same purpose.† In the longitudinal section of the anther the mother-cells will be found in various successive stages of development, which the observer will find very useful.

For a study of the development of the pollen mother-cell of the dicotyledons, we will take a plant from the *Ranunculaceæ* or the *Papaveraceæ*,—in this case *Helleborus foetidus*. In a floral bud which with its style measures about 8 or 10 mm. the successive anthers from within outward will give all the stages in the process of division. Treat the anther in the same way and with the same fixing and staining fluids as we did the *Fritillaria* and we shall get the same appearance only that the pollen grains are smaller. After the first step in the division of the mother-nucleus a cell plate will form among the connecting filaments, but again dissolve while the nucleus prepares for the second step. This fully agrees with the first in distinguishing the process from that of the *Fritillaria*. The nuclear pairs are united by connecting filaments. The four nuclei arrange themselves in the spherical mother-

*This difficulty may be obviated, however, by running a ring of shellac or other cement not soluble in the homogeneous fluid around the edge of the cover-glass.—A. B. H.

†Thus abbreviated the author. But very shallow shellac ring-cells put on with a turn-table and a hair pencil will serve all purposes much better.—A. B. H.

cell in the four corners of a tetrahedron, Fig. 113, *A*. Connecting fibres run freely in all directions through the cytoplasm between the four nuclei. Thus four new bundles of fibres are added to the two already existing in each of which is produced a cell-plate. The latter are distinct, but the connecting filaments are seen only under the most favorable conditions. The cell-plates have a circular quadrilateral form. They meet within the mother-cell. On the thick wall of the mother-cell are six inner, somewhat projecting ridges, *A*, to which the cell plates attach themselves with their outer edges. Cellulose walls are soon formed of the plates and the mother-cell is divided into four

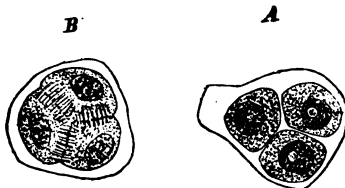


FIG. 113. *Helleborus fatiulus*. Pollen mother-cell at *A* in the act of dividing; at *B* fully divided. $\times 510$.

tetrahedrally-arranged daughter-cells. These four cells soon have walls of their own, while the mother-cell wall is dissolved.

Those plants in which cell-division was earliest observed belonged to the species *Cladophora glomerata*, whose structure we have already studied and know to be multinuclear (5). Cell-division is not necessarily preceded by a division of the nucleus. Each daughter-cell is provided with a number of nuclei which may further increase, so that the division of the cell and of the nucleus may go on independently of each other. Cell-division may be found going on at any hour of the day, but again we may often seek for it in vain. If one is found, others are likely to be in the same culture. One may easily recognize the process of division since the place where the division wall is to come is marked by a bright ring. The process (6) begins with a feeble annular collection of cytoplasm near the middle of the

length of the cell. The chlorophyll layer draws back correspondingly. The beginning of the division wall shows forth now as a sharp line. It projects ledge-like into the cell-space and presses the chlorophyll layer inward. The inconspicuous ring of cytoplasm remains on the inner edge of the projecting ledge. On both sides of the forming division wall cell-sap collects between the chlorophyll layer which is being pressed inward and the delicate membranous layer; thence the colorless ring in the dividing cell. The chlorophyll contents are finally bisected and the diaphragm-like wall fully closes up in the middle and makes a complete division wall. The chlorophyll contents remain for some time at some distance from the newly formed wall but gradually draw near. The nuclei are too small to allow the details of their division to be satisfactorily seen. The various steps in the division may be easily fixed with 1% chromic acid, but they are seldom met with.

All those processes of division of the nucleus which are connected with the formation of filaments are classed together as indirect nuclear division, and stand in opposition to the direct, which consists of a simple bisecting of the nucleus. The latter process is often seen in the older cells of the more highly organized plants,—as in the unusual case of the rapidly growing internodal cells of the *Characeæ* (7).

For the observation of the direct nuclear division of the older cells, the older internodes of *Tradescantia virginica* furnish a very favorable object. A longitudinal section examined in water will usually show a large number of them, Fig. 114, A. The nucleus exhibits its original contents but more or less irregularly contracted into several segments of different form and size. In many cases the pieces have been fully separated and lie together or at a

little distance from each other. There may be eight or ten of them of various sizes, and these again may increase by self-division. While the nucleus may be found in the act of division in almost all the elements of the section, it may be best observed in those of the medullary parenchyma. The thin-walled elements of the vascular bundles which have a nucleus show, besides, streaming protoplasm most beautifully. The nuclei may be quickly fixed with

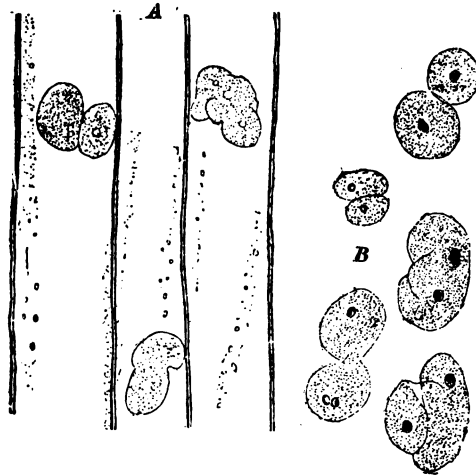


FIG. 114. *Tradescantia virginica*. Nuclei of the older internodes in the act of direct parting. *A*, in living state; *B*, after treatment with acetate of methyl-green. $\times 540$.

acetate of methyl-green when they come out very distinctly, Fig. 114, *B*.

Finally, taking our highest lenses to aid us, we will enter upon a question the solution of which is of the greatest moment for the complete understanding of plant bodies. It treats of the mutual connection of the protoplasmic cell bodies of the plant, which form a single continuous whole (8). Select *Rhamnus frangula* and having

removed the periderm from a stem about a centimeter thick make a delicate tangential section through the green rind. Put the section in water and direct attention to the chlorophyll-containing bast-parenchyma, which consists of rectangular cells tangentially extended. The walls are considerably thickened and provided with both large and small unbordered pits, the latter so narrow as to be scarcely distinguishable (9). Besides the bast-parenchyma the spindle-shaped groups of cells of the medullary rays are seen.

Now make a new section in the same direction, put it on a cover-glass and add a drop of concentrated sulphuric acid. After a few seconds immerse the whole in a glass full of water and wash the section thoroughly and quickly. Stain with an aqueous solution of aniline blue, wash with water and examine in dilute glycerine. Picric-aniline blue may advantageously be used instead of the other. Prepare it by dissolving to saturation picric acid in 5% alcohol and add aniline blue till the solution takes on a blue-green color.

Use the highest powers, a homogeneous immersion if possible. The effect of the acid is satisfactory if the walls of the bast-parenchyma are so much swollen that they show about the same diameter as the contracted cell body. The middle lamella is also swollen which makes the object still more favorable for our investigation. The contracted plasma body is beautifully stained with the aniline blue. The outlines of the single plasma bodies of the rind-parenchyma cells are smooth on those surfaces which border upon the cell walls which are provided with very fine pores. But when they adjoin walls with wider pits they are provided with processes more or less thick. These processes correspond in neighboring cells. If now we carefully examine the inclosing membrane between two particularly

broad oppositely-placed processes, we shall find a number of exceedingly fine, granular filaments stretched between them. They are the plasma threads by which living plasma bodies communicate with each other. The outer filaments of such a complex are bent and remind one strikingly of the connecting filaments which join two sister nuclei. Where the adjacent walls of two cells are smooth, we find that the middle layers of the cell wall are permeated throughout their whole extent by filaments which by a very considerable swelling of the cell wall will be separated from both the plasma bodies, but in case of less swelling will still be connected with them. These filaments are somewhat swollen and spindle shaped in the middle. In especially favorable cases the spindles will appear to be interrupted in the middle and the two halves connected by an extremely delicate granular thread. This appearance is seldom seen. Generally the plasma bodies do not all show us their common plasma connections, but rather those which have in no way been injured in cutting the section, and which have been suddenly fixed by the acid. The injured, or those not fixed with sufficient rapidity, have withdrawn their plasma filaments.

Those cell walls which are penetrated with fine filaments, have, with the filaments, an appearance which suggests the case of the nucleus- and cell-division where the connecting filaments served as starting points for the division wall, and which, now remaining intact, maintain a communication between the two cells (10). By the formation of broader pitted surfaces, the connection is afterwards maintained only within them, but that a direct connection exists between neighboring cells by means of plasma processes, seems to be now pretty well demonstrated.

It is much easier to demonstrate this proposition by some recently discovered facts involving the presence of proto-

plasmic masses in the intercellular spaces (11). For this investigation take a year old branch of *Ligustrum vulgare* (12). Put it in alcohol for some days in order to harden the cell body. Since by cutting a fresh object the cell contents might escape into the intercellular spaces, and vitiate the result, make a delicate section which shall include the primary rind and lay it in potassic iodide of iodine solution. The rind is formed of rounded, pretty-well-thickened cells with intercellular spaces of various sizes between. These are either filled or covered with a substance which takes the same yellow-brown color as the protoplasmic cell contents. The effect may be heightened if, after the removal of the iodine solution, one will add a little dilute sulphuric acid (acid 2 parts, water 1), which will produce a slight swelling, and the characteristic blue color of the walls. The yellow-brown color of the protoplasm in the cell and in the intercellular spaces will stand out very clearly. Still more instructive will be a longitudinal section, the cells being longitudinally elongated and some of the intercellular spaces of considerable length.

NOTES.

- (1) See for this lesson, Strasburger, Zellb. u. Zellth., III Aufl.; Flemming, Zellsbst. Kern, u. Zellth., Strasburger; die Controversen der Kerntheilung. In the latter work the literature.
- (2) Flemming, Arch. f. Micros. Anat., Bd. XIX, p. 317.
- (3) Flemming, Zellsbst. Kern, etc., p. 384.
- (4) For double staining of tissue these coloring substances were first proposed by J. Macfarlane, Trans. Bot. Soc. Edinb. Vol. XIV, p. 190.
- (5) Von. v. Mohl. im Jahre 1835 Dissert. Abgedr. in Flora 1837, ff.
- (6) Strasburger, work before quoted, p. 203.
- (7) Johow, Bot. Ztg., 1881, sp. 728, Strasburger; Ueber den Theilungsvorg. d. Zellk., p. 98, Auch. Arch. f. mikr. Anat. Bd. XXI. Literature there.

(8) For the general view, see Strasburger, *Bau u. Wachstum der Zellhaute*, p. 246, 1882.

(9) This object was recommended by Russow, the method of investigation by Gardiner, *Phil. Trans. Roy. Soc., Part III*, 1883, p. 821, ff.

(10) See Strasburger, work last quoted p. 248, and Russow *Stzber. d. Dorpater naturf. Gesell.* 1882.

(11) See Russow, l. c., p. 19, Berthold *Ber. d. deut. botan. Gesell.* II, Jahrg. p. 20.

(12) Recommended by Berthold, l. c.

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[Abbreviations: d = development, r = reaction, s = structure, u = use.]

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APPENDIX.

Staining with carmine.—Carmine solutions stain diffusely, but one may obtain a distinct color in the nucleus by laying the stained preparation for some time in 50% to 70 % alcohol containing 0.5 to 1 % muriatic acid, or in glycerine to which has been added 0.5 % muriatic acid.

Preparation of Beale's carmine.—Pour 2.3 cc. concentrated ammonia upon 0.6 g. pulverized carmine. After dissolving the carmine let it stand an hour and then pour it into a mixture of 66 cc. water, 47.5 cc. concentrated glycerine and 19 cc. absolute alcohol. Mix and filter after a short time.

Grenacher's alum-carmine.—Boil for ten to twenty minutes a 1-5 % aqueous solution of common alum with $\frac{1}{2}$ to 1 % of pulverized carmine and filter after cooling. To this add a trace of carbolic acid.

Grenacher's borax-carmine.—Dissolve 2-3 % carmine in a 4 % aqueous solution of borax and dilute with a like quantity of 70 % alcohol. Filter after a considerable time.

Thiersche's borax-carmine. Dissolve 4 parts borax in 56 parts distilled water. To this solution add 1 part carmine, and then mix 1 volume of the same with 2 volumes of absolute alcohol and filter.

Carminate of ammonia—Hoyer's neutral. Heat in a sand bath 1 g. carmine in about 1-2 cc. strong ammonia solution and 6-8 cc. of water, till the excess of ammonia has evaporated which is shown by the appearance of small bubbles and the fluid becoming a bright red. Filter the precipitate from the nearly neutral fluid after cooling, and to this add 4-6 volumes of strong alcohol. This causes the deposit of a bright red precipitate, which filter out and preserve. This powder is dissolved in water when needed and may be preserved by adding 1-2 % chloral hydrate.

Chlor-iodide of zinc.—Dissolve metallic acid in pure muriatic acid and evaporate to the consistency of sulphuric acid by the constant addition of the zinc. To this add all the potassic iodide that will dissolve and as much metallic iodine as it will take up.

Hoyer's mounting fluid.—For aniline preparations. Fill a wide-necked glass two-thirds full with selected white pieces of gum arabic and fill up the vessel with an officinal solution of potassic acetate or ammonia. By frequent shaking the gum will dissolve in a few days. Filter through blotting paper. For carmine or hæmatoxylin preparations a

several per cent solution of chloral hydrate to which has been added 5-10 % of glycerine should be substituted for the ammonia or the potassic acetate. If the fluid becomes turbid after a while it should be again filtered.

Glycerine-jelly.—To one part of the finest French gelatine, which has been soaked for two hours in six parts by weight of water, add seven parts of pure glycerine, and to each 100 g. of the mixture add 1 g. concentrated carbolic acid. Warm and stir for ten to fifteen minutes till all flocculence caused by the addition of the acid disappears. Finally filter while warm, through finest glass wool previously washed and still damp with distilled water.

Glycerine-gum.—10 g. gum Arabic, 10 g. water, 40-50 drops of glycerine.

Hæmatoxylin staining fluid, Boehmer's.—Dissolve 0.35 g. hæmatoxylin in 10 g. absolute alcohol, and to this solution add drop at a time from a solution of 0.1 g. alum in 30 g. distilled water, till a beautiful blue violet color is obtained.

Grenacher's.—No. 1, A saturated solution of hæmatoxylin in absolute alcohol. No. 2, A saturated solution of ammoniacal alum. Mix 4 cc. of No. 1 in about 150 cc. of No. 2. Let it stand a week in the light. Filter and add 22 cc. glycerine and 25 cc. methyl-alcohol. It should stand some time before using, to settle.

Cuprammonia.—The bright green precipitate, which cupric hyposulphate gives with dilute solution of ammonia, should be filtered and washed, and while still moist poured into concentrated ammonia, in which it dissolves with the development of heat. After cooling, crystals of hyposulphate of cuprammonia will form. The fluid contains only cuprammonia from which the crystals should be filtered out, and the solution kept in black bottles or in the dark.

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